2015
PATHOLOGY AND LABORATORY MEDICINE
RESEARCH DAY

MARCH 30, 2015

Program Guide

Schulich MEDICINE & DENTISTRY

Western

This event is an Accredited Group Learning Activity (Section 1) as defined by the Maintenance of Certification program of The Royal College of Physicians and Surgeons of Canada, and approved by Continuing Professional Development, Schulich School of Medicine & Dentistry, Western University (3.75 hours). Each physician should claim only those credits that he/she actually spent in the activity.

This program has no commercial support.

Message From The Chair

Our annual Pathology and Laboratory Medicine Research Day is one of the most rewarding days of the academic year. I would like to congratulate all the talented presenters for a fantastic job in representing the variety of research being carried out in the Department of Pathology and Laboratory Medicine. Our department is rich in talent, ideas, and research capabilities and this day showcases our activities and accomplishments in both basic and clinical science. This year, we have a record 60 presentations which is a great accomplishment for our relatively small but mighty department. While interacting with these scientists we can also get a glimpse of a fantastic future.

We are fortunate to have Dr. Nahum Sonenberg deliver the keynote address. Dr. Sonenberg is the James McGill Professor in the Department of Biochemistry at McGill University. He discovered the mRNA cap-binding protein, eukaryotic translation initiation factor 4E (eIF4E). Dr. Sonenberg has continued to explore the implications of his discovery and has provided critical insights into the role of eIF4E in health and disease. His ground-breaking work has also led to the discovery of eIF4E as a proto-oncogene. Dr. Sonenberg has received numerous awards including the Robert L. Noble Prize from the National Cancer Institute of Canada in 2002, the Killam Prize in 2005, the Gairdner International Award in 2008, the Centenary Award of the Biochemical Society (UK) in 2011, and the Lewis S. Rosenstiel Award in 2012. Most recently, Dr Sonenberg received the 2014 Wolf Prize in Medicine, one of the most prestigious awards.

The day would not be successful without the exceptional work of the organizing committee and many members of our department. I would like to personally thank Nancy Chan, Martin Duennwald, Manal Gabril, Zia Khan, Emily Goebel, Niamh Richmond, Jina Kum, Steffi Stephenson, Tracey Koning, Cheryl Campbell, Mellonie Carnahan, and Kathilyn Allewell. Lastly, I would like to thank the judges for interacting with our presenters, sharing their valuable experience, and offering insights. I hope you enjoy the day and learn about the fantastic research being carried out in our department.

Subrata Chakrabarti, MBBS, PhD, FRCP(C)
Chair, Department of Pathology and Laboratory Medicine, Western University
Chief, Department of Pathology and Laboratory Medicine, London Health Sciences Centre and St. Joseph's Health Care

2015
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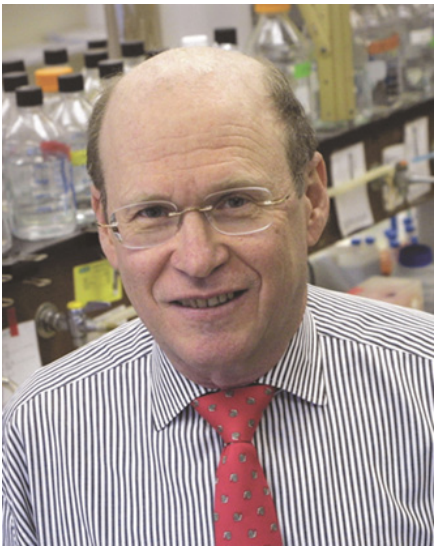
PROGRAM

9:05 - 9:15 am	**Welcome, Opening Remarks** **Dr. Subrata Chakrabarti** Chair/Chief, Pathology and Laboratory Medicine, Schulich Medicine & Dentistry, Western University and London Health Sciences Centre
9:15 - 10:15 am	**Keynote Speaker** **Dr. Nahum Sonenberg** James McGill Professor Department of Biochemistry, McGill University
10:15 - 10:45 am	**Nutritional Break**
10:45 - 12:15 pm	**Oral Presentations**
12:15 - 12:45 pm	**Faculty Presentation** Dr. Bekim Sadikovic Clinical Epigenomics: technology and applications
12:45 - 1:00 pm	**CME Evaluation and Group Photo**
1:00 - 4:00 pm	**Lunch, Poster Session and Networking**
4:00 - 6:00 pm	**Awards Ceremony** Windermere Manor

Featuring:

Dr. Nahum Sonenberg



James McGill Professor
Department of Biochemistry,
McGill University

Keynote Address:

Translational Control in Cancer and Autism

Platform Presentations

Time	Last Name	First Name	Title
10:45 am	Cecchini	Matthew	Loss of the Retinoblastoma Tumor Suppressor Leads to Improved Outcome in Patients with Lung Adenocarcinoma
11:00 am	Schick	Brian	Endocrine Cell Quantification May Help Distinguish Colorectal Hyperplastic Polyps from Sessile Serrated Adenomas
11:15 am	Zhang	Qi	Diagnostic Accuracy of Needle Biopsies in non-small Renal Masses and Development of a Renal Cell Carcinoma Xenograft Model
11:30 am	Milica	Krstic	The Role of Transcriptional Regulator TBX3 in Early Breast Cancer Progression
11:45 am	DiGregorio	Sonja	Deciphering the Role of RGNEF in ALS Using Yeast
12:00 pm	Kum	Jina	β-Adrenergic Receptor-dependent and -independent Mechanism of Propranolol in Infantile Hemangiomas

Platform Presentation #1

Loss of the Retinoblastoma Tumor Suppressor Leads to Improved Outcome in Patients with Lung Adenocarcinoma

Cecchini MJ[1,2,3], Ishak CA[1,2], Passos, DT[1,2,5], Palma, DA[4] Howlett CJ[3], Driman DK[3], Dick FA[1,2,5].

[1]London Regional Cancer Program, [2]Department of Biochemistry, Western University, [3]Department of Pathology and Laboratory Medicine, [4]Division of Radiation Oncology, [5]Children's Health Research Institute LHSC.

Introduction: The retinoblastoma tumor suppressor pathway is frequently inactivated in human cancer, enabling unrestrained proliferation. The majority of cancers; however, maintain expression of the retinoblastoma tumor suppressor protein (pRB). This protein is maintained in a hyper-phosphorylated state (ppRB) by mutation/alteration of upstream regulators, such as p16 and cylcin D. In addition to pRB's ability to regulate proliferation, it can control apoptotic signaling even in a hyper-phosphorylated state suggesting it can retain some function.

Methods: To test the clinical relevance of pRB status, we obtained tissue blocks from 93 cases of lung adenocarcinoma resected between 2003 and 2008. All cases received adjuvant platinum doublet chemotherapy. The median follow up for these cases was 4.6 years. All cases were stained for pRB and ppRB using immunohistochemistry and quantified based on intensity of staining and proportion of stained cells.

Results: We find that pRB expression is lost in 15% of lung adenocarcinoma cases. In tumors that do not express pRB, we found that the survival rate is significantly improved (HR, 0.37; 95% CI, 0.19-0.75) in comparison to tumors that express pRB. pRB status did not alter baseline levels of apoptotic or proliferative markers in these tumors.

Conclusions: This work identifies pRB as a novel marker in lung cancer, and its loss is associated with improved survival following adjuvant chemotherapy. This may be useful in classifying patients at greatest benefit for this therapy. Further understanding of this pathway is critical, as it may be possible to selectively target this pathway to sensitize pRB positive tumors to chemotherapy.

Keywords: Lung, adenocarcinoma, chemotherapy, pRB, E2F, immunohistochemistry

Platform Presentation #2

Endocrine cell quantification may help distinguish colorectal hyperplastic polyps from sessile serrated adenomas

Brian A. Schick, Jeremy R. Parfitt, Chaturika H. Herath, David K. Driman
Department of Pathology and Laboratory Medicine, London Health Sciences Centre and Western University

Introduction: Hyperplastic polyps (HP), sessile serrated adenomas (SSA) and traditional serrated adenomas (TSA) are colorectal polyps with major differences in clinical significance; SSAs and TSAs are premalignant while HPs are not, and SSAs and TSAs require removal followed by colonoscopic surveillance. These polyps can be morphologically difficult to distinguish. Studies have suggested that endocrine cells may be increased in HP, but absent or less numerous in SSA. We counted endocrine cells in these lesions to assess whether endocrine cell number is a useful discriminating feature.

Methods: Approval was obtained from the Tissue and Archive Committee of the London Laboratory Services Group. The London Health Sciences Centre database was searched for gastrointestinal biopsies diagnosed as HP, SSA, TSA, or serrated polyp unclassified (SPU) between December 1, 2010 and December 31, 2013, and 198 cases were evaluated. 80 cases were excluded due to a lack of well-oriented lesional crypts, excess cautery/crush artifact, an uncertain number of polyps present on individual slides, small polyp size (fewer than 5 contiguous lesional crypts present), and collision lesions. 118 cases were included: 51 SSA, 29 HP, 20 SSA with dysplasia (SSAD), 10 TSA, and 8 SPU. Immunohistochemistry for chromogranin A was performed on one block per case, and chromogranin-positive endocrine cells were manually counted in the lesional crypts.

Results: The mean number of endocrine cells per lesional crypt was 5.78 for HP, 2.50 for SSA, and 5.76 for SPU. In the SSAD, the mean number of endocrine cells per was 0.971 per non-dysplastic lesional crypt and 0.546 per dysplastic crypt. There were significantly fewer endocrine cells in SSAs compared to HPs ($p = 0.005$; unpaired t test).

Conclusions: Endocrine cells are less numerous in SSA compared to HP; quantifying them with chromogranin may be useful in morphologically difficult cases.

Keywords: endocrine cells, hyperplastic polyp, sessile serrated adenoma, traditional serrated adenoma, colon cancer

Platform Presentation #3

Diagnostic Accuracy of Needle Biopsies in non-small Renal Masses and Development of a Renal Cell Carcinoma Xenograft Model

Qi Zhang[1], Chantalle Willie[2], Jose Gomez[1], Hong Sing Leong[2], Nicholas Power[3]

[1]Department of Pathology and Laboratory Medicine, [2]Translational Prostate Cancer Research Group, [3]Department of Urology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada

Introduction: The role of percutaneous biopsy of small renal masses has been well established with diagnostic accuracy rates over 90% with high specificity. The accuracy of needle biopsies in larger kidney tumors has yet to be elucidated. The patient derived xenograft (PDX) on chick embryo's chorioallantoic membrane (CAM) model is an invaluable tool to study tumor development, metastasis and angiogenesis. No renal cell carcinoma (RCC) CAM model has yet been developed.

Methods: Six consecutive patients with renal masses greater than 6 cm undergoing radical nephrectomy in 2014 were recruited after REB approval and consent was obtained. An 18 gauge biopsy needle was used to sample the kidney tumor in 2X2 cm increments. The biopsies were sent for standard H/E staining, cell culture and CAM implantation. Pathologic interpretation was blinded. The biopsies and CAM PDX were recorded in terms of histologic type and Fuhrman grade (FG) if carcinoma was detected. The results were correlated with the final nephrectomy specimen.

Results: The median size of renal tumors that were biopsied was 8.8 cm (IQR 7.4 -9.2 cm). The median number of biopsies was 18 per specimen (IQR 13-23). Overall, 106 core biopsies were obtained. All renal tumors were determined to be clear cell renal cell carcinoma (ccRCC) on final pathologic interpretation. 56% of biopsies were determined to be ccRCC (56/106). Of the 56 biopsies determined to be ccRCC, the FG failed to correctly classify tumors into low (FG 1-2) or high (FG 3-4) in 41% (23/56) where the biopsy uniformly undergraded the tumors. 35% of the PDX were determined to be ccRCC (12/35). Similar to the biopsy, tumors were undergraded on the PDX.

Conclusion: While the role of renal mass biopsy in the diagnosis and management of small renal tumors is well established, caution must be used in interpreting results in larger tumors. There was excellent correlation of histologic subtype however but only 56% of biopsies were diagnostic. Furthermore, there was poor correlation of FG compared to the final pathology report. The RCC PDXs onplanted in the CAM of avian embryos is established in our lab and offer a robust platform for oncology research.

Keywords: renal cell carcinoma, Diagnostic Accuracy, Needle Biopsies, CAM, Xenograft

Platform Presentation #4

The role of transcriptional regulator TBX3 in early breast cancer progression

Milica Krstic[1,2], Ann F. Chambers[1,2,3], Alan B. Tuck[1,2,3]

[1]Department of Pathology and Laboratory Medicine, Schulich School of Medicine and Dentistry, Western University, London, ON, [2]The Pamela Greenaway-Kohlmeier Translational Breast Cancer Research Unit, London Regional Cancer Program, London Health Sciences Centre, London, ON, [3]Department of Oncology, Schulich School of Medicine and Dentistry, Western University, London, ON

Introduction: Our recent studies show that breast cancer cells that have gained the ability to invade adjacent tissue express high levels of the regulatory protein TBX3. In two cell lines derived from the same breast cancer patient at different phases of cancer progression (21T series), TBX3 is abundant in the invasive 21MT-1 cell line, while being minimally expressed in the non-invasive, DCIS-like 21NT cell line.

Methods: Both TBX3 isoforms were overexpressed in 21NT cells and knocked down in 21MT-1 cells. Cells were grown in 3D Matrigel; colony characteristics and Ki67 and caspase 3 levels were quantified. In vitro migration and invasion assays were conducted. Cells were injected into the chick embryo chorioallantoic membrane (CAM) vasculature to examine in vivo extravasation potential and invadopodia formation. Gene expression changes associated with TBX3 isoform overexpression were examined using qRT-PCR arrays.

Results: Overexpression of TBX3 isoforms in 21NT cells resulted in increased survival, growth and invasiveness in vitro. Cells overexpressing TBX3 had higher extravasation efficiency as well as functional invadopodia formation in vivo in the CAM model. Extravasation and invadopodia formation were reduced by ~80% and ~40%, respectively, with TBX3 knockdown in invasive 21MT-1 cells. This was also accompanied by reduction in colony size in vitro.

Gene expression changes associated with TBX3 isoform up-regulation were examined, and suggest that TBX3 promotes the transition from in situ to invasive breast cancer through the altered expression of key regulatory and EMT-related genes, which are common to both isoform transfectants. Differences in gene expression were also noted between the isoforms. Interestingly, in preliminary findings, TBX3iso1 overexpressing cells have increased tumorigenic potential in nude mice.

Discussion: TBX3 isoforms promote progression of DCIS-like 21NT cells. This work may have clinical potential in identifying patients with high-risk lesions, and/or as potential direct or indirect therapeutic targets to prevent disease progression.

Keywords: TBX3, breast cancer, metastasis, ductal carcinoma in situ (DCIS), invasive mammary carcinoma (IMC), epithelial-mesenchymal transition (EMT)

Platform Presentation #5

Deciphering the Role of RGNEF in ALS Using Yeast

Sonja Di Gregorio and Dr. M. Duennwald

Department of Pathology and Laboratory Medicine, Western University

Introduction: Amyotrophic lateral sclerosis is a devastating motor-neuron disease. Our research will focus on a newly discovered protein called rho guanine nucleotide exchange factor (RGNEF). Recent biochemical and pathological studies strongly implicate RGNEF as an ALS protein yet the underlying mechanism remain unknown.

Hypothesis: Rho Guanine nucleotide exchange factor (RGNEF) contributes to ALS pathogenesis by modulating protein misfolding and protein aggregation.

Materials and Methods: We are the first to establish an RGNEF yeast model to evaluate the role of RGNEF in ALS. RGNEF must therefore be expressed in yeast and these yeast strains characterized regarding RGNEF toxicity, localization, and aggregation. RGNEF toxicity in yeast has been evaluated using yeast growth assays. Using our yeast model and cultured mammalian cells we aim to determine how RGNEF modulates the misfolding, aggregation, and toxicity of other ALS proteins and thus explore the role of RGNEF in modulating protein misfolding in ALS.

Results: We have shown that RGNEF when expressed in yeast is mildly toxic, resulting in lower growth of the cells expressing WT RGNEF as well as some of the other constructs. Furthermore, our studies show that RGNEF interacts with TDP-43 and FUS, both of which are well-established ALS proteins. Both TDP-43 and FUS have been previously expressed in yeast and are toxic. We have shown that co-expression of these proteins with RGNEF alleviates toxicity compared to vector controls.

Summary and Discussion: We have established a yeast model expressing RGNEF. Our model will allow the exploitation of powerful yeast genetic and biochemical tools to systematically investigate RGNEF misfolding, subcellular localization, and its role in ALS.. Additional work in higher model systems is required to fully characterize these interactions and their role in ALS.

Keywords: RGNEF, ALS

Platform Presentation #6

β-adrenergic receptor-dependent and -independent mechanism of propranolol in infantile hemangiomas

Jina Kum[1] and Zia A. Khan[1,2,3]

[1]Department of Pathology and Laboratory Medicine, Western University, London ON, [2]Metabolism and Diabetes Research Program, Lawson Health Research Institute, London ON, [3]Division of Genetics and Development, Children's Health Research Institute, London ON

Introduction: Infantile hemangioma is the most common tumour of infancy. Currently, propranolol, a β-blocker commonly used for cardiac complications, is the most effective pharmacological intervention for hemangiomas. We have shown that hemangiomas arise from multipotential stem cells (hemangioma-initiating cells; hemSCs). Although propranolol causes apoptosis of vascular endothelial cells (ECs), its role in hemSCs is unknown. Unfortunately, recent reports suggest that up to 20% cases of hemangiomas regrow upon cessation of propranolol treatment. Since some hemangiomas regrow following propranolol treatment, we hypothesize that the hemangioma-initiating cells are not responsive to propranolol.

Methods: Primary stem cells isolated from human hemangiomas were cultured in the presence of propranolol. Normal vascular ECs were used as controls to determine selectivity of propranolol action.
Results: Our results revealed that normal vascular ECs predominantly express β1-receptor subtype, whereas hemSCs express β2 and β3. This profile was related to the contrasting responses whereby ECs showed apoptosis upon propranolol exposure but not hemSCs. Furthermore, propranolol did not alter downstream signaling mediators of β-adrenergic receptors in hemSCs. Similarly, use of specific β1- and β2-adrenergic receptor siRNA did not significantly affect the cell number in hemSCs, indicating a possible alternative mechanism. Upon further screening, we show that hemSCs expressed serotonin receptor (5-HT7), which was 40-fold higher as compared to normal ECs. Using receptor agonists, we reveal that activation of 5-HT7 receptor mimics the effects of propranolol. Furthermore, specific inhibition of 5-HT receptor normalized the effects of propranolol, confirming the involvement of 5-HT receptors in propranolol's action in hemSCs.

Conclusions: Our study reveals that propranolol may have distinct effects in the different cell types found in hemangioma. Specifically, our findings show that propranolol mediates its effect in hemangioma through altering serotonin receptors in hemSCs and not β-adrenergic receptors. These findings may lead to novel and fast-acting treatment targets for life-threatening hemangiomas.
Keywords: Infantile hemangioma, propranolol, stem cells, endothelial cells, β-adrenergic receptor, serotonin receptor

Poster Abstracts

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6	Crombie	Samantha	The Pathology Tissue and Archive Committee: Its Role in Human Tissue Research
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10	Filek	Richard	Investigating the Structural and Functional Changes to the Retina Following PRP in Diabetic Retinopathy Patients
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12	Garcia-Marquez	David	Internal Consultation Practice for Endometrial Hyperplasia
13	Goebel	Emily	Transforming Tumour: A case of a Metastatic Nonseminomatous Testicular Tumour with a Unique Histological Profile
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19	Kanagalingam	Tharsan	Determining the Effect of Glucocorticosteroid Treatment on CRTH2 Expression Levels in Th2 Cells
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Poster Abstract #1

Hajed Alharbi[1, 2], Rabindra N. Bhattacharjee[1, 3], Manujendra N Saha[1, 3], Patrick PW Luke[1, 2, 3]

[1]Matthew Mailing Centre for Translational Transplant Studies, Lawson Health Research Institute, [2]Department of Pathology and Laboratory Medicine, Western University, [3]Department of Surgery, Western University, London, Ontario

Introduction: Ischemia reperfusion injury (IRI) is an unavoidable consequence in kidney transplantation. It is the major cause of reducing graft survival after transplantation. IRI is the result of inflammation and subsequent damage caused mainly by innate immune receptors called Toll Like receptors (TLRs). Damaged cells from ischemic allograft release molecules including high-mobility group box 1(HMGB1) and heat shock proteins known as damage -associated molecular patterns (DAMPs) which can be recognized by TLRs to promote inflammation. Stimulation of different TLRs induces distinct patterns of gene expression, which not only leads to the activation of innate immunity but also led to the development of acquired immunity. In this study, we hypothesize that TLR(s) signaling may play an important role in renal IRI and targeting its pathway would a significant impact in the prevention of IRI and allograft damage.

Methods: Macrophages cell line RAW264.7 and bone marrow derived dendritic cells were stimulated by lipopolysaccharide (LPS) with or without an anti-inflammatory agent called Carbon monoxide releasing molecule (CORM 401). Expression of TLRs , proinflammatory cytokines, and DCs maturation markers were then tested by qRT-PCR, Flow Cytometry, ELISA and Western Blot analysis. To mimic in vivo IRI situation, we have generated a C57BL/6 mouse model by renal pedicle clamping for an hour followed by 24 h of clamp release. To see the effect of CORM401 in vivo, CORM 401 were injected (i.p.) 1 or 18 hour before clamping .Kidneys were collected, histological and biochemical analysis were done.

Results: Preliminary data demonstrates that mostly TLR3, 4, 7 and 9 are involved in the IRI processes and responsible for the production of chemokines, and cytokines usually observed in IRI

Conclusion: Allograft damage and side effect of immunosuppression are two major problems in kidney transplant recipient. Thus, any therapy, which specifically targets TLR, has potential application in the prevention of IRI and prolongation of renal allograft life time.

Keywords: Kidney, Ischemia Reperfusion injury, Toll like receptors, Innate immunity, carbon monoxide

Poster Abstract #2

Understanding the relative contributions of neoblast cells and differentiated cells to planarian regeneration

Michelle Anderson[1], Taisaku Nogi[2,3], Michael Thwaites[4], Zia A. Khan[1,5], and Thomas Drysdale[2,3]

[1]Department of Pathology and Laboratory Medicine, Western University, [2]Department of Physiology and Pharmacology, Western University, [3]Department of Paediatrics, Western University, [4]Department of Biochemistry, Western University, [5]Diabetes Research Program, Lawson Health Research Institute

Introduction: Fundamental knowledge on the nature of stem cell regeneration and morphogenesis is important for understanding the development and maintenance of all organisms. The planarian has emerged as a novel model to address these issues, due to its large population of stem cells, termed neoblasts, that are necessary for cell renewal and regeneration of missing tissue. Previous research using planarians has demonstrated differentiated muscle cells maintain the body pattern during regeneration by communicating with the neoblasts. This study aimed to understand neoblast and differentiated cell interactions that determine body morphology and degree of pigmentation. We hypothesized that the differentiated cells will determine the morphology and that the neoblast cells will determine the degree of pigmentation.

Methods: To examine this question, we exploited the neoblasts' ability to restore the body of an organism that has lost its neoblasts. We attempted to restore neoblasts in one species with neoblasts from another species with a different morphology. The host species, Dugesia japonica, was irradiated to eliminate endogenous neoblasts, and then rescued with neoblasts from another species, Schmidtea mediterranea.

Results: Our preliminary results indicate that the rescue neoblasts are capable of proliferating and migrating within the host species because the neoblasts have taken proper positioning within the host. In addition, qualitatively there appears to be an increased degree of pigmentation in the host after neoblast introduction. The neoblasts and differentiated cells appear to be incapable of cell-cell communication as attempts at regeneration are unsuccessful.

Conclusions: These initial findings suggest that the degree of divergence between the two species is substantial enough to prevent effective neoblast and differentiated cell interaction.

Keywords: Neoblast cells, differentiated cells, body morphology, pigmentation, planarians

Poster Abstract #3

Psammomatous Melanotic Schwannoma: Cytomorphologic, immunohistochemical, and electron microscopical features

Saeed Asiry[1,2], Lee-Cyn Ang[1,2] and Keith MacDougall[3,4]

[1]Department of Pathology and Laboratory Medicine, Western University, [2]Department of Pathology and Laboratory Medicine, London Health Sciences Centre, [3]Department of Clinical Neurological Sciences, Western University, [4]Division of Neurosurgery, London Health Sciences Centre

Introduction: psammomatous melanotic schwannoma is a rare tumor with about 50% of patients have associated Carney's complex with myxomas (heart, skin, and breast), spotty pigmentation of the skin, and endocrine over activity. The behavior of the tumor is difficult to predict because local recurrence and metastasis may occur even in the absence of malignant features

Case: The patients was 27 years old lady, suffered from neuropathic pain along the dorsal aspect of her right leg for 3 months as well as from two episodes of urinary incontinence. Imaging diagnostics revealed a right S1 nerve root tumor explaining the aforementioned symptoms. Intraoperative smears showed a tumor composed of epithelioid cells with round to oval nuclei, few prominent nucleoli and intracytoplasmic brown pigmentation. Scattered calcifications are also noted. Electron microscopy show well-defined and thickened pericellular basal lamina with pigmented melanosomes of variable stages of development (including premelanosomes).

Discussion: Tumor imaging plus the presence of calcifications can be helpful to consider psammomatous melanotic schwannoma during intraoperative consultations. Imunnohistochemistry is of limited value in such case because the tumor was S100, HMB-45, and Melan-A positive. Electron microscopy is the only definitive tool that can differentiate this tumor from other mimickers (e.g. melanoma "primary and metastatic" or spinal meningeal melanocytoma).

Keywords: Cytology, melanotic schwanoma, electron microscopy, psammomatous, calcification

Poster Abstract #4

The role of aberrant Nrf2/Keap1 pathway activation across a broad spectrum of human cancers

Shannon Baker, Dr. Martin Duennwald and Dr. Chris Howlett

Department of Pathology and Laboratory Medicine, Western University

Keap1 is a negative regulator of the transcription factor Nrf2, which is activated during times of oxidative stress. Keap1 mutations have been identified in non-small cell lung cancer, yet over activation of Nrf2 and the potential role of Keap1 mutations in other cancer types have not been systemically assessed. Our goal is to investigate numerous different types of cancer for the presence of these Keap1 mutations and for the up-regulation of Nrf2. We will also investigate the molecular mechanism by which overactive Nrf2 can contribute to the cancer phenotypes. We have already explored cases of endometrial cancer, renal cell carcinoma and head and neck cancers. We tested these cases for the upregualtion and nuclear localization of Nrf2 as well as localization of Keap1 using immunohistochemistry. We also use yeast models to explore the cellular mechanisms underpinning Nrf2 activation. Yeast cotransformed with the cancer-related Keap1 mutants and Nrf2 have been analyzed to give insights into toxicity associated with over active Nrf2. We expect that the mutant Keap1 proteins will fail to interact with and de-activate Nrf2.

Keywords: cancer, oxidative stress, antioxidant, chemotherapy, reactive oxygen species, Nrf2, Keap1, immunohistochemistry, yeast model

Poster Abstract #5

Expression of Human Tissue Kallikreins (KLKs) in Polymorphous Low Grade Adenocarcinoma (PLGA)

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Introduction: Polymorphous low grade adenocarcinoma (PLGA) is the second most common malignant salivary gland tumor of the minor salivary glands. Human tissue kallikreins (KLKs) are a family of highly conserved serine proteases expressed by various tissues throughout the body. KLKs have become powerful tumor markers for the diagnosis of the cancer patient (e.g. PSA (KLK3)). The literature demonstrates a link between KLKs and salivary gland neoplasms.

Objective: To determine levels of KLK mRNA in tissue samples of formalin fixed paraffin embedded polymorphous low grade adenocarcinoma (PLGA). Secondly, we wish to determine if KLK expression is limited to tumor cells alone.

Methods: Nineteen cases of PLGA were reviewed (1987-2013). A diagnosis of PLGA was confirmed, demographic data was collected, and formalin fixed paraffin-embedded PLGA and normal salivary gland tissue samples were obtained. RNA isolation was achieved, followed by conversion to complementary DNA via reverse transcription. Synthesized DNA primers were added to target kallikrein DNA and through PCR, the quantitative level of expression of KLKs 1-15 was recorded. Samples exhibiting high and low KLK expression were selected for immunohistochemistry staining, using a standard protocol.

Results: In total, 19 samples of PLGA were obtained from the archives at the Department of Pathology, Western University. RNA isolation yielded 0.32ng of RNA from 16/19 PLGA tissue samples. Preliminary PCR data reveals an increase in the mean KLK (1-15) mRNA expression in all of the PLGA tissue samples, as compared with normal salivary gland tissue. KLK1, KLK4, KLK10, KLK12, and KLK15 showed statistically significance(Mann Whitney U test, $p<0.05$). Immunohistochemistry results demonstrate tumor specific staining.

Discussion/Conclusion: KLK mRNA demonstrates an increase expression in tissue samples of polymorphous low grade adenocarcinoma. Furthermore, the tumor cells stain positively and specifically for kallikreins.

Keywords: Polymorphous low grade adenocarcinoma, kallikreins, salivary gland neoplasia

Poster Abstract #6

The Pathology Tissue and Archive Committee: Its Role in Human Tissue Research

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Introduction: Human tissue is invaluable to medical research and its nature demands consideration of a number of perspectives and regulations. Pathology departments are charged with balancing these interests and responsibilities to ensure optimal patient care while facilitating medical research. In response to the increasing volume, complexity, and expense of requests for tissue for research the Pathology Tissue and Archive Committee (TAC) was established to manage all applications and disbursements according to best practices.

Methods: Since 1999, TAC applications have been tracked in a database. TAC processes and data were retrospectively reviewed alongside relevant legislation, research ethics guidelines and the scientific literature. TAC user feedback was separately obtained via voluntary survey.

Results: In its first 15 years the TAC reviewed 895 applications. Oncology (44%) was the highest user, LHSC (32%) the most common institutional affiliation and NCIC (11%) the most frequent funding source. The most recent year saw 75 new studies involving over 2500 tissue blocks. Estimates revealed an average cost recovery of $1333 per study; less than 1.2% of respective grant budgets. Survey results confirmed that users found TAC operations to be consistently in the satisfactory to excellent range.

Discussion: The TAC supports research involving human tissue by creating a secure tissue repository and a standardized process for vetting requests. The TAC has come to play a central role in coordination, communication and education while applying best practices towards tissue handling and patient privacy. Tracked data provides valuable insight into a number of important activities for an academic department: optimization of processes, objective prediction of expenditures, reimbursements, usage patterns and funding sources. The data further underscores the magnitude of research facilitated by Pathology and Laboratory Medicine and the need for appropriate stewardship of tissue resources.

Keywords: Pathology, Laboratory Medicine, Tissue, Research, Legislation, Ethics, Stewardship

Poster Abstract #7

Expression of Human Kallikrein Protein and RNA in Maxillofacial Cysts and Tumours

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Introduction: Non-inflammatory odontogenic cysts and tumours vary in clinical appearance and growth potential and can cause widespread destruction and deformation of the face. Proteases may play a role in the differing pathogenesis of these cysts and tumours. Human kallikrein proteins (KLKs) are a group of 15 serine proteases implicated in a variety of signalling and regulatory roles including as tumour suppressors in breast and gastric cancer. KLK over-expression is associated with cancer/neogenesis. This study evaluates common neoplasms and cysts of odontogenic origin, the lateral periodontal cyst, dentigerous cyst, keratocystic odontogenic tumour (KOT) and ameloblastoma for the presence of specific KLKs. Secondarily, we evaluated ameloblastoma tissue for the expression of KLK mRNA.

Methods: Archived paraffin embedded tissue samples were obtained, cut and assessed for the presence of KLKs using a standard immunostaining technique utilizing antibodies for KLK 3, 4, 5, 9 & 11. Analysis of epithelial immunostaining was performed utilizing a scoring system assessing staining intensity as well as proportion of cells stained. RT-PCR was utilized to evaluate KLK 1-15 mRNA expression in pooled samples of ameloblastoma tissue.

Results: Immunostaining revealed the presence of KLK 3, 4, 9 & 11 in all tissue types studied. Greater KLK 3 staining was present in ameloblastomas and KOT's than in the odontogenic control. KLK 9 exhibited greater staining in KOTs and dentigerous cysts than the odontogenic control. KLK 11 had greater staining in the ameloblastomas than in non-odontogenic cystic controls. KLK 5 was present only in KOT's. Expression of KLK 1, 4, 7, 8, 10 & 12 mRNA was found in pooled ameloblastoma tissue.

Conclusion: KLK 3, 4, 9 & 11 are present in the six maxillofacial cysts and tumours investigated. KLK 5 is present only in the keratocystic odontogenic tumour. KLK 1, 4, 7, 8, 10 & 12 mRNA were identified in the ameloblastoma.

Keywords: Human kallikrein proteins, biomarkers, prostate specific antigen, odontogenic tumours, odontogenic cysts, oral and maxillofacial pathology, head and neck pathology, immunostaining, protein expression, RNA expression

Poster Abstract #8

A Canada-wide survey of the use of Surgical Pathology Grossing Templates and their Application at London Health Sciences Centre through Dragon Speech

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Introduction: The use of surgical pathology grossing templates is an effective way of limiting pathologist assistant (PA) error, assuring quality of and standardizing gross dictations, highlighting and uncluttering crucial information for pathologists, and improving specimen turnaround time. Currently, London Health Sciences Centre (LHSC) has grossing templates which are formatted in a cluttered and lengthy manner and are not accessible by Dragon. The aim is to gather existing grossing templates in order to modify, develop and implement templates through Dragon for all surgical specimens at LHSC.

Methods: A PubMed literature review was performed to find published grossing templates for surgical pathology. Four of the main pathology governing bodies (American Association of Pathologists Assistants, College of American Pathologists, American Society for Clinical Pathology, and Canadian Association of Pathologists) were contacted for standardized grossing templates. Individual hospitals, health authorities and diagnostic laboratories across Canada were contacted for unpublished, site-based grossing templates currently in use at their institution. The collected grossing template examples were reviewed and modified to best suit the protocols, specimen types and preferences at LHSC.

Results: Literature searches for published grossing templates proved negative, however, general formatting and style of dictations was reported on and concluded the same ideas. The governing bodies provided many online resources for grossing protocols but not standardized templates. Thus far, more than 40% of the contacted hospitals, authorities and laboratories have replied and about 40% of those created and use their own grossing templates for biopsies and surgical specimens.

Discussion: It can be concluded that there are no published standardized grossing templates for surgical specimens and the majority of institutions have created their own templates to facilitate their grossing needs. Once LHSC has created and implemented grossing templates, future surveys will be distributed in hopes to gather feedback on the format, time-consumption and effectiveness of the templates from the PAs and pathologists.

Keywords: grossing, templates, dictation, surgical pathology, pathologists' assistants (PAs), Dragon Speech

Poster Abstract #9

Ontario Growth Standards for Infants: A Retrospective Autopsy Study – Study Updates

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Background: Statistics Canada predicts on average 747 infant deaths per year in Ontario based on birth and infant death databases from 1993 to 2008. A postmortem examination is required in a significant number of these deaths to determine the cause and manner of death. In these cases, the pathologist will compare the autopsy findings to standardized population parameters using body and organ measurement charts. The reference resources are based on autopsied populations. Although many resources are available, many are outdated and have significant limitations.

Objectives: To create Ontario population-specific organ and body measurement mean charts for infants and neonates under one year of age; growth graphs for each age category; and predict organ weights based on body weight, sex and age.

Methods: Data were collected from 1260 Coroner's files from the archives of the Office of the Chief Coroner for Ontario which investigated the deaths of children under the age of one year for the period of 2000-2010. Recorded data included various quantitative and qualitative characteristics of body and organs and relevant pathological findings. To assist in creation of a new standard resource, a survey of Ontario pathologists and literature review were undertaken.

Results: The literature review and survey identified 20 reference sources used by Ontario pathologists in the course of performing pediatric autopsies. The survey determined these references to be outdated and limited in scope. Guidelines were proposed to assist in creating a new Ontario population autopsy body and organ measurement resource.To date, data on organ and body measurements are currently being analyzed.

Keywords: Infants; Neonates; Growth Standards; Autopsy; Age; Body Measurements; Organ Weight.

Poster Abstract #10

Investigating the Structural and Functional Changes to the Retina Following PRP in Diabetic Retinopathy Patients

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Introduction: Diabetic retinopathy is the leading cause of blindness worldwide and is estimated to affect more than 100 million adults. Over the past 30 years, epidemiological studies and clinical trials have shown that timely laser photocoagulation could prevent vision loss. Laser therapy has been well proven to stabilize and control proliferative disease. Questions however on the long-term effects on the nerve fiber layer and optic disk from these treatments remain to be answered. The objective of the study is to evaluate retinal changes using structural and functional diagnostic tests at defined period of times up to 24 months in diabetic retinopathy patients.

Methods: Patients will undergo pre-laser and 3,6, 12 and 24 month post-laser visual fields, Heidelberg Retinal Tomograph (HRT) and Optical coherence tomography (OCT) tests. OPTOS fluorescein angiography will be performed at pre-laser baseline, 6, 12 and 24 months post-laser.

Results: From baseline (n=20), average retinal nerve fiber layer (RNFL) thickness did not change significantly (P>0.05) by 12 months post-treatment (3 months +2.4 µm; 6 months +3.5 µm; 12 months -2.8 µm). A non-significant thickening of the macula was observed (+5.0 µm) at 3 months, (+ 3.3 µm, P>0.05) at 6 months and 12 months (+6.2 µm) compared to baseline. Visual field MD decreased non-significantly compared to baseline (12 months -1.19 dB).

Discussion: 12 month analysis indicates that laser is a safe treatment and does not cause structural and functional changes to the retina and optic nerve. Further correlation will be made as patients continue to be recruited and undergo their 24 month testing.

Keywords: Diabetic retinopathy, ophthalmology, retina, optic nerve, panretinal photocoagulation

Poster Abstract #11

Novel Technique for Correlation Between in Vivo MRI and Histopathology for Prostate Cancer and its Mimickers

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Prostate Cancer (PCa) is the most common non-cutaneous cancer in North American Men. Early detection increases the number of treatment options and improves the survival rates. The Trans-rectal ultrasound-guided biopsy is the current accepted standard for diagnosing this cancer. It has around 40% rate of over- or underestimating the Gleason grade. The Multi Parametric MR Imaging (MPMRI) has revealed promising results in mapping PCa and the surrounding tissue. It can be used as non-invasive procedure to predict the locations and prognosis of PCa. Radiologists identify and contour potential lesions according to Prostate Imaging Reporting and Data Systems (PI-RADS). The MRI signaling to non-cancerous abnormalities that could mimic PCa in imaging prostate has not been tested in correlation with histopathology digital imaging.

The Co-registration of the image findings with the histopathology analysis of PCa is a crucial step. In our study we aim to develop a novel technique for correlation between in vivo MRI and histopathology for PCa and its mimickers. Therefore, Radical prostatectomy specimens from LHSC marked with 10 standard-shaped fiducial markers per specimen after fixation with formalin. The external fiducials (lamb kidney) socked in Magnevist. The internal fiducial (cotton embroidery Floss) socked in Magnevist and blue dye. They used as a landmark in histology processing and MRI. Then the whole gland imaged according T1 and T2 weighted 3T MRI protocols followed by slicing at 4.4 mm intervals then processed for whole-mount histology sections. Initial registration between fiducial markers on histology and MR images has been performed. Based on initial registration we developed a special digital technique for registration of in vivo to ex vivo MRI with digital histopathology images.

The result of this study will help increase the accuracy of detecting PCa and play a major role in diagnosis and classifying the confounder that mimics cancer in MR images.

Keywords: Prostate imaging, 3D prostate reconstruction, prostate MRI, prostate registration, in vivo MRI

Poster Abstract #12

Internal Consultation Practice for Endometrial Hyperplasia

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Background: We sought to review our internal consultation practice for endometrial hyperplasia and assess any needed changes to our practice, especially in the light of the new two tier WHO classification for endometrial hyperplasia.

Design: We reviewed the internal consultations for endometrial hyperplasia for 2010 to determine: (1) frequency of consultation; (2) reasons for consultation; (3) agreement between original and consultant pathologists and (4) consultation rates for each team member of the team and; (6) tendency to adhere to team guidelines. As well, an internal anonymous survey was circulated to gather information about individual practices for endometrial hyperplasia.

Results: For 244 cases of endometrial hyperplasia, there were 80 (33%) internal consultations. The two commonest reasons for consultation were: 1) presence or absence of atypia in complex endometrial hyperplasia (40%), and 2) differentiation between atypical hyperplasia and well differentiated endometrioid adenocarcinoma (39%). Agreement between original and consultant occurred in 75% of consultations with interpretation differences in 25%. The main reason for interpretation differences was the presence or absence of atypia. The consultation rates varied from 4-31% and may not necessarily be associated with case volume. Most team members saw the process as time consuming and selected a consultant based on availability and seniority.

Conclusion: Our internal consultation practice for endometrial hyperplasia will likely not be impacted by the two tier WHO classification since the commonest reasons for consultation remain important in the new classification. Given the high overall internal consultation rate and interpretation differences, endometrial hyperplasia appears to be a continual problematic diagnosis. Further study seems necessary to investigate the discrepancy in internal consultation rates between pathologists.

Keywords: endometrial hyperplasia, internal consultation.

Poster Abstract #13

Transforming Tumour: A case of a Metastatic Nonseminomatous Testicular Tumour with a Unique Histological Profile

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Introduction: The most common type of nonseminomatous germ cell tumour (NSGCT) is a mixed tumour with a combination of histologic types. Metastatic testicular cancer to the retroperitoneal lymph nodes (RPLN) is common. There can be a histological discordance between the primary testicular tumour and the metastasis. We report a case of a late recurrent metastatic mixed NSGCT with two uncommon features that convey poor prognosis: choriocarcinoma and somatic-type malignancy.

Case: A 29 year old male presented with recurrent testicular cancer in the RPLN following a 4 year remission post orchiectomy and chemotherapy. He originally presented with a testicular mass, slightly elevated AFP and normal HCG. The primary tumour was a mixed NSGCT, predominantly embryonal carcinoma with teratomatous and yolk sac components. He presented 4 years later with gynecomastia and elevated HCG. The histologic profile of the metastatic tumour was different from the primary and consisted of choriocarcinoma, teratoma and neuroendocrine neoplasm, a somatic-type malignancy.

Discussion: To our knowledge this is the first case reported with these two poor prognostic features in recurrent metastatic testicular cancer. Choriocarcinoma in a metastasis from a primary tumour that does not contain a choriocarcinoma component is very rare. In a review of 100 cases comparing primary and metastatic tumours this did not occur once, and in another study of 61 cases of metastatic germ cell tumours of the testis only one case was reported as having a metastatic choriocarcinoma component with no choriocarcinoma component present in the primary tumour. In addition, the frequency of somatic type malignancies occurs in only 3-6% of testicular tumours, with only 50% of those occurring only in the metastatic component. Only one other case of neuroendocrine neoplasm (carcinoid tumour) arising from a testicular teratoma has been reported.

Keywords: Testicular neoplasm, germ cell tumour, somatic malignant transformation, embryonal carcinoma, choriocarcinoma, teratoma, late recurrence

Poster Abstract #14

Intra-departmental quality assurance program for pathologists' assistants in surgical pathology

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Introduction: Due to the uniqueness of each specimen and the vast fluctuation in complexity, the quality of gross examination in surgical pathology, performed by Pathologists' Assistants (PAs), or other equivalent health professionals, has historically been a hit-and-miss and an ongoing challenge. The current quality assurance (QA) program at London Health Sciences Centre (LHSC) has limited success on assessing the quality of sections and dictations, and does not monitor the productivity. Hence, as a multiannual project, an improved program needs to be created. In this study, we aim to review the currently available QA for PAs, in order to establish foundation for a reliable and standardizable QA program.

Methods: A PubMed literature and textbook review was performed to find published QA for PAs. Governing bodies in North America, Australia and United Kingdom, such as Canadian Association of Pathologists, College of American Pathologists, were contacted for guidelines. Then over 190 hospitals, quality organizations and laboratories across North America were contacted for unpublished QA programs currently being applied in their institution. Finally the data collected was analyzed to shape a new QA program.

Results: To our best knowledge, there are no publications or textbooks that describe a QA program for PAs in surgical pathology. However, some contents can be used as criteria for assessment. The governing bodies provided no guidelines. Over 25% contacted institutions provided feedback. Criteria were analyzed to form the new QA program.

Discussion: While there are no published QA programs for PAs, the awareness is raising across Canada. Many institutions have been innovative and created unique programs to assure the PA's quality. From the pooled and selected criteria and ideas, we will be able to invent a reliable and standardizable QA program which can be beneficial to all surgical pathology institutions.

Keywords: Pathologists' Assistant (PA), surgical pathology, quality assurance (QA), productivity, gross examination

Poster Abstract #15

The Role of Malat-1 in Diabetic Complications

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Introduction: It is documented that only about 2% of the human genome codes for proteins; however, nearly the entire genome is transcribed. Among the classes of noncoding RNAs are the long non-coding RNAs (lncRNAs) which include metastasis associated lung adenoma transcript1, or malat-1, a 7kb RNA molecule made up of a single exon, and highly conserved in 33 mammalian species. Furthermore, its baseline levels of transcription are extremely high. Malat1 is associated with increased metastasis and angiogenesis in several cancers but still its function has not been determined. Malat1 has not been studied in the context of diabetic complications. A recent study by our lab using human endothelial cell culture has shown that malat1 expression is increased under exposure to high glucose as compared to normal glucose levels. Based on this we hypothesized that malat1 plays an important role in the pathogenesis of chronic diabetic complications.

Methods: We will use streptozotocin to induce diabetes in some wild type mice as well as transgenic Malat-1 knockout mice. We aim to characterize malat1 transcript levels in multiple tissues in the diabetic mouse over a 3 point time course of 2 weeks, 1 months and 2 months. We further aim to looking look at the histology and expression of specific downstream proteins and markers of inflammation such as interleukin 6 and tumour necrosis factor α in the tissues with the greatest changes in transcript abundance to examine specific tissue changes.

Results: Wild type and transgenic MALATKO mice have been successfully made diabetic and will be monitored for blood glucose levels and body weight until specific time points of study.

Conclusions: The data generated from this study will provide direct evidence as to whether Malat1 is important in the pathogenesis of chronic diabetic complications. We will also know whether it can be used as a potential treatment target.

Keywords: Malat-1, Long non-coding RNA, Diabetic Complications, Genetic model, Mice

Poster Abstract #16

Characterization of ALDHhi/CD133+ Cells in the Human Fetal Pancreas

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Introduction: Understanding how endocrine progenitors commit to a β-cell lineage during human fetal development is necessary for the design of in vitro β-cell differentiation protocols. Previous research has determined that high aldehyde dehydrogenase (ALDH) activity is necessary for the development and survival of β-cells. The purpose of the current study is to assess the endocrine lineage commitment of ALDHhi/CD133+ human fetal pancreatic cells and determine their potential for forming a pool of endocrine cell precursors.

Methods: Human fetal pancreata (18-22 weeks) were dissociated to a single cell suspension, labeled for ALDH and CD133, and sorted into double positive (ALDHhi/CD133+) and double negative (ALDHlo/CD133-) populations using fluorescence-activated cell sorting (FACS). Sorted populations were than cultured for an extended period to assess their phenotypic stability and then characterized using immunofluorescence staining and qRT-PCR to quantify various transcription factors and cellular markers indicative of endocrine cell commitment.

Results: Fluorescence-activated cell sorting yielded a larger population of double positive cells than double negative cells. Expression of ALDH and endocrine-specific transcription factors was not detected in either cell population after extended cell culture. Both of the expanded populations were positive for vimentin, Ki-67, CK19, SOX9 and β-catenin, with no significant differences in expression levels.

Conclusions: Our preliminary results indicate that neither population is capable of maintaining expression of ALDH or endocrine-specific markers after extended culture. Further studies should assess each population's capacity for forming islet-like cell clusters to further characterize any underlying differences in endocrine lineage commitment.

Keywords: Aldehyde dehydrogenase, CD133, diabetes, β-cell, stem cells, FACS, human fetal pancreas

Poster Abstract #17

miR-200b regulates Fibronectin expression in Renal Endothelial Cells of Mice with Diabetic Nephropathy

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Introduction: Diabetes is the leading cause of end-stage renal failure in North America. Endothelial cell dysfunction is a key component in the development and progression of diabetic nephropathy. Expression of fibronectin (FN) by endothelial cells is increased in diabetes, and contributes to basement membrane thickening and mesangial matrix expansion observed in diabetic nephropathy. We have previously shown that microRNA-200b (miR-200b) plays an important role in diabetic retinopathy. Furthermore, based on bioinformatics analyses, FN is a target of miR-200b. Hence, in this study we examined whether miR-200b is important in the pathogenesis of diabetic nephropathy and whether such effects are mediated through regulation of FN production.

Methods: Transgenic mice were engineered to overexpress miR-200b under the control of an endothelial cell specific Tie2 promoter. Diabetes was induced in wild-type and transgenic C57Bl/6 mice using low dose streptozotocin. The animals were monitored with respect to body weight and blood glucose levels. Mice were sacrificed after two months of diabetes and kidneys were harvested. Tissue from the renal cortex was analysed for FN mRNA and protein expression.

Results: Diabetic mice had reduced body weight and hyperglycemia. Analyses of endothelial cells isolated from the kidneys showed significantly increased miR-200b expression in the transgenic mice compared to wild-type controls. We observed a statistically significant increase in FN mRNA in the kidneys of diabetic mice compared to wild-type controls. Both basal and diabetes-induced increases in FN were abrogated in the diabetic mice overexpressing miR-200b.

Discussion: Our data suggests that miR-200b overexpression may protect against increased extracellular matrix production in diabetic nephropathy. Future experiments will be performed to determine the mechanisms of such regulation by miR-200b.

Keywords: MicroRNA, miR-200b, fibronectin, endothelial cells, diabetic nephropathy, extracellular matrix proteins

Poster Abstract #18

Loss of IgG4 expression is associated with recurrence of primary sclerosing cholangitis

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Introduction: Primary Sclerosing Cholangitis (PSC) is a chronic progressive disease of unknown etiology. Pathologically, PSC is associated with lymphoplasmacytic infiltrate around the bile ducts. It has been shown that increased IgG4 positive infiltrate are present in IgG related sclerosing diseases morphologically similar to PSC. The aim of the current study were to determine a) whether increased IgG4 positive cells are present around the large duct in end-stage PSCs and b) whether such changes are associated with post transplantation recurrence of PSC.

Method: Immunohistochemical stainings for IgG4 were performed on a total of 79 explanted livers. All surgeries were done at the London Health Sciences Centre, between 1990 to 2014. All stains were carried out on the blocks taken from the porta-hepatis. IgG4 positivity were scored as mild (10-29 cells/HPF), moderate (30-50 cells/HPF) or marked (>50 cells/HPF). Recurrence of PSCs were confirmed by imaging studies and/or liver biopsy.

Results: PSC recurrence occurred in 15 out a total of 79 cases. Among the 64 non-recurrent PSCs, nine (14 %) cases showed mild positivity, 2 showed (3.1 %) moderate positivity and one demonstrated (1.5 %) marked positivity. In contrast, only one of 15 (6.6 %) explants from the patients with post-transplant PSC recurrence, showed mild IgG4 positivity. Chi-square analyses confirmed significant difference of IgG4 positivity in these two groups.

Conclusion: Data from this study suggest that reduced IgG4 positivity is associated with increasing likelihood of PSC recurrence.

Keywords: Primary sclerosing cholangitis, IgG4

Poster Abstract #19

Determining the Effect of Glucocorticosteroid Treatment on CRTH2 Expression Levels in Th2 Cells

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Introduction: The chemoattractant homologus receptor for Th2 cells (CRTH2) is responsible for mediating chemotaxis of inflammatory cells in an allergic response. Specifically in Th2 associated asthma, exposure to an allergen in the lung mucosa can result in increased CRTH2 activation. Glucocorticosteroid therapy is aimed to minimize the allergic response in asthmatics by reducing cytokine production and inflammatory cell recruitment. The regulation of CRTH2 expression in Th2 cells in response to glucocorticosteroid treatment is currently not well understood. This study aims to determine the effect of the glucocorticosteroid, dexamethasone, on CRTH2 expression in Th2 cells. We hypothesize that dexamethasone treatment of Th2 cells will result in increased transcription of CRTH2 in Th2 cells.

Methods: CCRF-CEM cells will be cultured and treated with 1-10μM of dexamethasone for 6, 24, and 48 hours to determine the effect it has on CRTH2 mRNA expression. CRTH2 mRNA transcript expressed in CCRF-CEM cells will be quantified using quantitative real time polymerase chain reaction (qRT-PCR).

Results: Using in silco analysis we have identified $CRTH2^{+}$ and Th2 specific expression in the CCRF-CEM cell line. An in vitro assay has been developed to determine changes in CRTH2 expression in CCRF-CEM cells in response to dexamethasone treatment.

Conclusion: The results of this experiment will provide novel results on the effect of dexamethasone treatment on CRTH2 mRNA expression in Th2 cells. Our results may provide an incentive to further investigate a CRTH2 mediated mechanism of glucocorticoid resistance exhibited in severe asthmatic patients.

Keywords: Th2 Cells, CRTH2, Glucocorticosteroid, Dexamethasone, Severe Asthma, Allergic Disease

Poster Abstract #20

Risk Management and the Autopsy; The Autopsy Checklist

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Introduction: Any autopsy has defined risks in the pre-autopsy, autopsy and post-autopsy phases. The medical literature suggests that these risks be managed in a standardized manner. The London Health Sciences Centre Department of Pathology & Laboratory Medicine has an Autopsy Checklist to address these risks. Our goal was to assess the effectiveness of this checklist in documenting certain risks since its implementation in 2012.

Methods: Communication of infectious risk by pathologists was the focus of the study. This risk was assessed by examining autopsy reports, noting predetermined risk factors and comparing this information to the risks noted on the checklist. Compliance was determined by comparing the total number of cases to the number of checklists completed. Any additional comments were noted to ensure no other previously unidentified risk was present.

Results: Infectious risk factors were recorded more in the narrative autopsy report than in the Autopsy Checklist. Although all risk factors were underreported on the checklist, prostitution and HIV were the most significant with 100% and 75% of cases being unrecorded. Compliance for checklist completion was highest among the forensic pathologists (2012 to 2013 -92% to 93%). The neuropathologists and autopsy team pathologists showed compliance rates of 75% and 64% in 2012, changing to 61% and 64% in 2013. Of the 11 sharps injuries, Pathologist's Assistant students sustained 36%.

Conclusion: The checklist does provide a standardized means of recording the communication of infectious risks. This study shows the effectiveness of the checklist depends on its accurate completion by the case pathologist. Pathologist's Assistant students and their supervisors need to be vigilant about safe autopsy practices to avoid injury.

Keywords: autopsy checklist, risk management, sharps injury, infectious risk

Poster Abstract #21

Expression of Kallikrein-related Peptidases (KLKs) in Adenoid Cystic Carcinoma

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Purpose: Kallikrein-related peptidases (KLKs) are a group of 15 serine proteases implicated in a wide variety of biological processes. Overexpression of KLKs has been associated with the development of certain cancers, and epithelial-mesenchymal transitions leading to metastasis. Furthermore, expression of specific KLKs is associated with unfavourable clinical outcomes in a number of different cancers. The clinical application of KLK 3, known as prostate specific antigen, as a biomarker highlights the potential clinical utility of KLKs in the diagnosis, prognosis and surveillance of tumors. However, the role of KLKs in salivary tumors has not been extensively studied. The purpose of this study was to determine whether dysregulated gene expression of KLKs occurs in adenoid cystic carcinomas (ACC). The consequence of altered expression will be investigated to determine its usefulness in predicting tumor behaviour and guiding the therapeutic management of cancer patients.

Methods: Formalin-fixed, paraffin-embedded (FFPE) tissue specimens were obtained from the Oral Pathology archives of Western University and London Health Sciences Centre. Total RNA was then extracted from a total 40 FFPE samples, which included 25 adenoid cystic carcinomas and 15 from normal salivary tissue. Complementary DNA, obtained by reverse transcription, was then combined with gene specific kallikrein primers (KLK1-KLK15) to allow for quantitative real-time PCR. Data was normalized to a β-actin housekeeping gene.

Results: Normal salivary tissue and adenoid cystic carcinomas both express all 15 KLKs. However, ACC showed a significant decrease in the expression of KLK 1, 8, 11, 14 (Mann Whitney U-value, $p<0.05$).

Conclusion: The expression of KLKs in adenoid cystic carcinomas differs from that of normal salivary tissue. These results are consistent with the aberrant expression of kallikreins in other cancers. Previous research in our laboratory has shown a downregulation of KLK 1, 11, 14 and an upregulation of KLK 8 in mucoepidermoid carcinoma. Also, higher protein levels of KLK 14 were identified in ACC compared to normal salivary tissue using immunohistochemistry. Further research will focus on the possibility of a negative feedback loop whereby KLK protein levels and/or protein function decreases gene expression.

Keywords: kallikreins, adenoid cystic carcinoma, biomarker, salivary cancer, RT-PCR, gene expression, formalin fixation and paraffin embedding (FFPE)

Poster Abstract #22

Mucosa-Associated Lymphoid Tissue Lymphoma Mimicking an Upper Gastrointestinal Polyp

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Case: An 88-year-old man underwent upper gastrointestinal (GI) endoscopy for intermittent dysphagia and mild aspiration. This revealed what was thought to be a benign upper GI polyp. One year later he re-presented complaining of worsening symptoms and 15-20 pounds of weight loss. Laryngoscopy confirmed the presence of a large polypoid mass arising from the post-cricoid area of the hypopharynx, and the patient subsequently underwent transoral endoscopic resection. Frozen section on the resected tissue showed a lymphoid proliferation, and further analysis confirmed this to be extranodal marginal zone lymphoma of mucosa associated lymphoid tissue type (MALT lymphoma).

Discussion: The hypopharynx is an uncommon location for the presentation of MALT lymphoma, and it is infrequently reported in the literature. Nevertheless, it remains an important consideration in the differential diagnosis of polyps and masses in this and other mucosa-lined sites.

Keywords: MALT lymphoma, hypopharynx, post-cricoid, laryngoscopy, polyp

Poster Abstract #23

Injury Patterns Sustained in Motor Vehicle Collisions with Driver's Third Generation Airbag Deployment

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Introduction: Following introduction of first generation airbags and consequent fatalities due to their "aggressive" nature of deployment, second and later third generation airbags were developed. While there are studies describing injuries associated with first and second generation airbags, no studies have documented injuries associated with third generation airbag deployment.

Materials: Fatal driver Motor Vehicle Collision (MVC) case files, which occurred in the year 2012, were retrieved from the Ontario Office of the Chief Coroner and reviewed. Inclusion criteria: collisions had to be full frontal or offset frontal; drivers had autopsies documenting injuries. Model of vehicle was used to determine the airbag generation which deployed during the collision. Odds ratio statistical analysis was used.

Results: In total 295 MVC were reviewed and 65 cases met the inclusion criteria of the study. Craniocerebral, cervical spinal, thoracic and abdominal injury patterns were not statistically different for third generation airbag deployments when compared to first/second airbag deployments or airbag non-deployment cases. Seatbelt use did not affect injury patters among third generation airbag deployment collisions. When all 65 collision cases were analyzed, 17 cases sustained a combined injury pattern to thorax and abdomen, and 15 sustained a set of craniocerebral, thoracic and abdominal trauma. Most of the motor vehicle collision cases were of high impact severity (most 80 km/h and above).

Conclusions: Airbag deployment, regardless of generation, and seatbelt usage play little role in determining injury patterns in drivers killed in the collisions analyzed. High speed appears to be the critical factor in determining injury patterns.

Keywords: airbags, collisions, injuries, pathology, third generation

Poster Abstract #24

Solitary Fibrous Tumour of the Lacrimal Sac Presenting with Recurrent Dacryocystitis: Case Report and Literature Review

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Introduction: Solitary fibrous tumour is a rare spindle cell neoplasm that frequently arises in the pleura and mediastinum (1). This tumour has, however, been reported in numerous other locations and may be misdiagnosed due to variable clinical presentations and histological appearances. We present a case of solitary fibrous tumour of the lacrimal sac.

Case: A 44-year-old woman presented with excessive tearing and a slow growing soft mass in the lacrimal sac fossa. Ultrasound revealed a hypoechoic, solid tumour. Histopathological examination revealed a moderately cellular, spindle cell neoplasm with prominent vascularity. The cells, admixed with thick collagen bands, were arranged in fascicle. The tumour cells exhibited minimal pleomorphism. Necrosis and mitotic figures were not identified. Immunohistochemically, the tumour cells were positive for vimentin and CD34 and were negative for S-100. A Reticulin stain confirmed the presence of reticular fibers between tumour cells

Discussion: Lacrimal sac tumours are rare. They are generally described as proliferation of bland-looking spindled to oval epithelioid cells that form short fascicles or clusters, admixed with collagen bands, and a prominent branching vasculature. To the rest of our knowledge, only two cases of solitary fibrous tumour have been published in the literature. The prognosis of solitary fibrous tumour is difficult to predict. The tumour may recur locally following incomplete excision.

Keywords: Lacrimal sac tumour. Solitary fibrous tumour. Recurrent Dacryocystitis

Poster Abstract #25

Grading of Total Mesorectal Excision Specimens: Inter-rater Variation in Assessment

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Introduction: Total mesorectal excision (TME) is the 'gold standard' procedure for the surgical treatment of rectal cancer. Examination of the quality of the TME specimen in the pathology department is an important prognostic factor as this correlates with local recurrence and overall prognosis. Using a TME grading form, specimens were assessed in both fresh and formalin-fixed states. The objective was to determine if there was variation in grading between assessors and between fresh and formalin-fixed specimens.

Methods: The raters included surgeons, pathologists, pathologist's assistants (PAs), a resident and a PA student. Prior to fixation, each specimen was assessed fresh by up to 6 raters. The specimen was then prepped and fixed in formalin for 96 hours before being assessed again by 2 PAs. The 4 parameters evaluated were: mesorectal bulk, surface regularity, presence of defects and coning.

Results: A total of 13 TME specimens have been evaluated thus far. The inter-rater agreement was measured using the Fleiss kappa statistic and varied across all parameters. Fresh specimens were rated by up to 6 raters and assigned an overall grade. The Fleiss kappa scores for parameters 1-4 and overall grade were 0.268, 0.436, 0.531, 0.216, and 0.467 respectively. Inter-rater agreement of the parameters for fresh specimens among 2 PAs was poor (kappa < 0.333) for all parameters. The agreement among 2 PAs for grading post formalin fixation was fair to very good for all parameters (kappa values ranging from 0.549-1).

Conclusions: The results indicate variability between assessors, with some parameters yielding better inter-rater agreement than others. This study highlights the need for additional knowledge-transfer, to ensure good consistency and accurate grading of TME specimens.

Keywords: total mesorectal excision, pathological assessment, rectal cancer, quality of surgery, pathology

Poster Abstract #26

Is Perineural Invasion in Prostatic Core Biopsies Prognostically Significant?

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Introduction: The prognostic significance of perineural invasion (PNI) in prostate cancer (Pca) is debated. PNI is defined as the presence of cancer tracking along or around a nerve. Since PNI is a major mechanism of Pca extension from parenchyma to periprostatic soft tissue, PNI which is sampled on prostate biopsy may signal an increased likelihood of extraprostatic extension (EPE) of cancer. The objective of the study is to evaluate the significance of PNI in the prostate biopsy in predicting EPE/ high pathological stage (specifically pT3a) in RP.

Methods: 389 prostatic core biopsies and their corresponding RP specimens performed at the LHSC were included (2010 to 2013). The cases were divided into “group 1” and “group 2”: 188 and 201 cases with negative and positive PNI biopsy respectively. Clinicopathological data (age, PSA level, Gleason score (GS), % of tumor volume, number of positive specimens in biopsy, presence or absence of EPE (focal/non-focal) and PNI) was collected.

Results: 65/188 (34.5%) cases from “group 1” and 136/201 (67.6%) cases from “group 2” showed positive EPE in the corresponding RP. 106/136 (79.7 %) cases in “group 2” had non-focal EPE (p= <0.001). PNI was significantly associated with EPE. Furthermore, the cases were categorized into 4 groups: PNI - /EPE – (n=123), PNI - /EPE + (n=65), PNI + /EPE – (n=65) and PNI + /EPE + (n=136). There was a significant difference in GS, tumor volume and number of positive specimens between the 4 groups (p<0.001).

Discussion: The presence of PNI in core biopsy is significantly associated with EPE in RP. PNI can be a significant predictor for high pathological stage (pT3a) in RP.

Keywords: prostate cancer, perineural invasion, extraprostatic extension, biopsy, radical prostatectomy

Poster Abstract #27

Prolongation of Cardiac Allograft Survival through Targeted Silencing of TLR Adaptor Genes using Mannose Liposome

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Introduction: MyD88 and TRIF, the common adaptors of Toll-like receptors (TLRs) signaling, evoke dendritic cells (DCs)-mediated antigen-specific immune rejection. Knockdown of two distinct downstream pathways in DCs may abolish TLR-induced adaptive immune response, thus preventing rejection of cardiac grafts in transplantation. We hypothesized that mannose-conjugated liposome with small interference RNA (siRNA) can specifically deliver siRNA to DCs that abundantly express mannose receptor; gene silencing of MyD88 and TRIF genes in DCs can specifically block TLR adaptor pathway and may prolong allogeneic heart graft survival.

Method: Mannose liposomes were made in order to incorporate siRNA specific forMyD88 (Man-siMyD88) and TRIF siRNA Man-siTRIF). Recipients (BALB/c mice) were treated with Man-siMyD88 + Man-siTRIF, 3 and 7 days prior to heart transplantation and 7, 14, 21 days after transplantation. Control groups were injected with mannose liposome with scramble siRNA (Man-siGl2) and non-targeted liposome without mannose (lipo-siMyD88 and lipo-siTRIF). After siRNA treatment, a fully MHC-mismatched (C57/BL6 to BALB/c) heart transplantation was performed.

Results: A significant prolongation of allograft survival was observed in the recipients treated by Man-siMyD88 + siTRIF (mean survival time MST=63.5 days). In contrast, MST of allogeneic hearts in recipients treated with control Man-siGl2 or non-targeted lipo-siMyD88 + siTRIF was 5.8 days and 15.7 days respectively. The prolongation of allograft survival is associated with immunomodulation induced by generation of tolerogenic DCs and Treg cells. Additionally, lower levels of INF-γ, IL1-β and higher levels of IL-10 and IL-6 were shown in an MLR when using DCS isolated from treated recipients, suggesting that targeted silencing of TLR pathways in DCs promoted Th differentiation.

Conclusion: This study demonstrated a new method of DC-specifically targeted siRNA delivery in vivo using mannose-conjugated liposomes, which specifically deliver siRNA to the spleen DCs, knock down TLR adaptors, and prolong allograft survival, implying the potential of a novel and effective RNAi-based anti-rejection therapeutics in heart transplantation.

Keywords: TLR adaptors, heart transplantation, RNAi, Mannose

Poster Abstract #28

Microvascular endothelial cells undergo RIPK3 independent, CD4+ T-cell mediated death in chronic cardiac allograft rejection

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Introduction: Necroptosis is a form of programmed necrotic cell death that is dependent on receptor-interacting protein kinase (RIPK) 1 and 3 and can be induced by death-receptor ligation with various cytokines. Our previous study found that RIPK3-mediated necroptosis regulated microvascular endothelial cell (MVEC) death in acute rejection. This study aims to establish the role of RIPK3 in CD4+ T cell-mediated chronic rejection by using the single MHC class II mismatch [C57BL/6 (H-2b) to bm12 (H2-Ab1bm12)] transplantation model.

Methods: Cell death in-vitro was induced by cytokine death treatment or CTL (cytotoxic T lymphocytes) and necrosis was measured by flow cytometry and SYTOX® Green Nucleic Acid staining on the IncuCyte ZOOM. Released HMGB1 protein was measured by immunobot. Hearts were transplanted from C57BL/6 wild type and B6;129R1-RIPK3tm1Vmd (RIPK3 null) into Bm12 mice and followed by histological examination.

Results: Flow cytometry and SYTOX® Green staining showed TNFα-induced necroptosis in MVECs was enhanced with SMAC-mimetics and zVAD-fmk, and confirmed by Necrostatin-1 inhibition of RIPK1. Necroptosis was significant at all tested time points. Interestingly, no significant differences were found between CTL-induced cell death in wild type and RIPK3 null MVECs. Endothelium thickening and intima narrowing, hallmarks of CAV and chronic rejection, were found in both wild type and RIPK3 null cardiac grafts within 60 days, and no significant difference was found between days survival of wild type and RIPK3 null hearts.

Conclusions: Contrary to our previous study that showed RIPK3-mediated necroptosis deficiency protected MVECs in acute cardiac allograft transplantation; RIPK3 does not seem to have an effect on CD4+ T cell-mediated cell death. Next, we will study the mechanisms of CD4+ T cell-mediated death in wild type and RIPK3 null MVECs after cardiac allograft transplantation.

Keywords: necroptosis, cardiac transplantation, RIPK3, CD4+ T-cells, microvascular endothelial cells

Poster Abstract #29

Expression of Growth Hormone Secretagogue Receptor 1a and Ghrelin in Human Heart Failure

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Introduction: Currently the prognosis for heart failure (HF) remains poor and there is no single diagnostic test for HF. The development of new cardiac-specific biomarkers for HF could greatly improve HF diagnosis. Our study aimed to investigate whether myocardial levels of growth hormone secretagogue receptor 1a (GHS-R1a) could potentially serve as a biomarker for HF.

Methods: A previously characterized ghrelin analog, Cy5-ghrelin (1-19), was used to assess GHS-R1a levels in the right atrium (RA) of explanted and implanted hearts of 2 transplant patients, and of samples from 5 cardiac surgery patients with varying severities of HF. Ghrelin levels in the RA of transplant and surgery patients were also assessed using fluorescence immunohistochemistry.

Results: Levels of both GHS-R1a and ghrelin were significantly increased in diseased explanted hearts as compared to healthy implanted hearts of transplant patients ($p \leq 0.0001$). Levels of GHS-R1a were significantly increased in 3 surgery patients in comparison to healthy implanted hearts of transplant patients ($p \leq 0.01$). Levels of ghrelin were significantly increased in all 5 surgery patients in comparison to healthy implanted hearts of transplant patients ($p \leq 0.001$).

Discussion: Our study represents the first quantitative findings of significantly increased GHS-R1a and ghrelin levels in the RA of explanted hearts as compared to healthy implanted hearts of transplant patients. In contrast to our initial hypothesis, these findings suggest a parallel increase in both GHS-R1a and ghrelin levels in HF. Our study encourages further research aimed at understanding ghrelin system changes in HF and future potential use of GHS-R1a as a biomarker for HF.

Keywords: Heart failure, biomarkers, ghrelin, growth hormone secretagogue receptor, GHS-R1a, Cy5 ghrelin (1-19), fluorescence

Poster Abstract #30

Growth Differentiation Factor 15 has a Protective Role in LPS-induced Acute Kidney Injury

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Introduction: Septic acute kidney injury (AKI) is a leading cause of morbidity and mortality with no accepted method of therapy. Growth differentiation factor 15 (GDF15) is induced in many diseases, however its role in Lipopolysaccharide (LPS)-induced AKI is unknown. We aim to determine the role of GDF15 in LPS-induced AKI to assess its therapeutic potential. As GDF15 is known to have anti-inflammatory and anti-apoptotic effects, we hypothesize that GDF15 has a protective effect in LPS-induced AKI in the mouse model.

Methods: GDF15 knockout (KO), transgenic (TG) and wild-type (WT) mice received 4mg/kg injections of LPS. Renal function was determined using serum BUN and creatinine levels. Kidney tissues were stained with H&E and scored based on area of tubular damage. Apoptosis and neutrophil infiltration were also detected by the TUNEL and MPO assays, respectively. The expression of inflammatory mediators was measured by quantitative RT- PCR.

Results: LPS treatment induced AKI as evidenced by the increased levels of BUN and serum creatinine. Our results show GDF15 KO mice treated with LPS had the highest levels of BUN and creatinine. In contrast, GDF15 TG mice had significantly lower BUN and creatinine levels compared to KO, indicating that GDF15 has a protective effect in LPS-induced renal injury. GDF15 KO mice generally had greater levels of tubular damage, as indicated by swollen epithelia and luminal precipitate in histological sections, compared to GDF15 TG mice. We also found that GDF15 deficiency in KO mice augmented the expression of TNF-α and MCP-1 compared to WT mice.

Conclusion: Our results show that GDF15 may play a protective role in LPS-induced acute kidney injury, potentially through inhibiting the NFκB pathway. We continue to elucidate the underlying mechanism by examining other inflammatory mediators at both the mRNA and protein level and through in-vitro studies.

Keywords: Septic acute kidney injury, LPS, nephrotoxin, GDF-15, NAG-1, inflammation

Poster Abstract #31

Cation-Permeable Channel TRPM7 is found in the Ganglion Cell Layer of the Mouse Retina

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Introduction: Recent evidence has indicated that ischemia may play a role in Retinal Ganglion Cell (RGC) death that is characteristic of open-angle glaucoma. Ischemia can be brought on by a variety of factors such as ocular hypertension which is found in 50% of glaucoma presentations, as well as other pre-disease states. Transient receptor potential melastatin member 7 (TRPM7) is a non-selective cation-permeable channel that conducts ions such as Ca2+ and Mg2+ across the plasma membrane, and has been implicated in neuronal death following transient ischemia-reperfusion. TRPM7 is up-regulated shortly after hippocampal ischemia-reperfusion, and conducts a large positive current into the cell in ischemic conditions. This study will investigate the role of TRPM7 in the retina. We hypothesize that TRPM7 is expressed in RGCs and plays a role in cell death following ischemia-reperfusion.

Methods: This study employed immunofluorescence on sagittal sections of young adult mouse eyes to localize TRPM7 to retinal cell layers with particular focus on the ganglion cell layer. We then harvested retinas and cultured primary mixed retinal cells for fluorescent immunocytochemistry and confocal analysis of TRPM7 expression in particular cell types.

Results: Our immunofluorescence studies on sagittal eye sections of wild-type mice show that TRPM7 is expressed in the retina. TRPM7 localizes to the ganglion cell layer, as well as the inner and outer plexiform layers of the retina. Confocal analysis of primary retinal cultures shows that ganglion cells are present in the cell culture.

Conclusions: These preliminary findings pave the way for further studies which will investigate whether TRPM7 plays a role in ganglion cell death. Future experiments will test the effects of acute and chronic oxidative stress on ganglion cell death, and examine whether TRPM7 inhibitors increase cell survival under these conditions.

Keywords: Retina, glaucoma, ganglion cells, TRPM7, ischemia

Poster Abstract #32

Epithelial to mesenchymal transition in the metastatic progression of gastroenteropancreatic neuroendocrine tumors

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Introduction: Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are a rare type of malignant epithelial cancer arising from the diffuse neuroendocrine system. In this study, we have performed gene expression profiling along with immunohistochemistry (IHC) to highlight key molecular pathways underlying the pathogenesis and metastasis of GEP-NETs. We hypothesized that epithelial to mesenchymal transition is involved in the pathogenic progression of GEP-NETs.

Materials and Methods: Formalin-fixed paraffin-embedded archived tissue samples of NETs arising from small intestine (SI-NETs), pancreas (P-NETs), and colorectal region (C-NETs) were used to extract RNA for real-time PCR-based gene profiling, and to construct tissue microarrays (TMAs) for high-throughput immunohistochemistry with antibodies against B-catenin, E-cadherin, vimentin, SMADs and Snail/Slug.

Results: Gene expression results showed elevated VEGF receptor and ligand expression patterns, variations in expression patterns of ECM remodelling genes, an abundant TGFBR1 and SMAD2 expression which point to global cellular behavior associated with epithelial to mesenchymal transition (EMT) and cell guidance. In our IHC studies with antibodies against E-cadherin, β-catenin, and vimentin, a subset of cases from the TMAs display an expression pattern akin to an EMT phenotype where there is a loss of E-cadherin and β-catenin expression in the membrane along with strong vimentin reactivity.

Summary and Discussion: Given that GEP-NETs tend to present with metastasis, EMT likely occurs at some point along the disease driving progression and malignancy. Our results show evidence of EMT at the mRNA and protein levels. From the genes highlighted by our gene expression studies, TGF-β signalling seems to be a key mediator towards this EMT event. Therefore, we will proceed to investigate whether TGF-β signalling is active in GEP-NETs and determine whether its activation is linked to an EMT phenotype.

Keywords: Gastroenteropancreatic neuroendocrine tumours, epithelial to mesenchymal transition, gene expression, tissue microarray, immunohistochemistry

Poster Abstract #33

Iron-dependent ferroptosis mediates microvascular endothelial cell survival following cardiac allograft transplantation

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Introduction: The field of programmed cell death (PCD) has recently been expanded to include novel forms of regulated necrosis (RN), which have been implicated in various disease models. One modality of RN, known as iron-dependent ferroptosis, has recently been implicated in renal ischemia reperfusion injury (IRI), but has yet to be studied in a transplantation model. Since cardiac endothelial cells undergo ischemia and reperfusion before and after transplantation, we aimed to demonstrate that ferroptosis is involved in solid organ cell death and therefore cardiac allograft rejection.

Methods: Murine microvascular endothelial cells (MVECs) and tubular epithelial cells (TECs) were cultured and treated with the ferroptosis inducer erastin. We will apply ferroptotic inhibitors and iron chelators to measure the degree of protection conferred, and eventually study this pathway in an in vivo mouse model of heterotopic heart transplantation.

Results: Following treatment with erastin, necrotic cell death was higher in murine TECs compared with untreated TECs in non-proliferative conditions. Similarly, MVECs undergo ferroptosis following erastin treatment compared with untreated MVECs.

Discussion: Renal TEC have been shown to be highly susceptible to ferroptosis compared to other forms of RN in an IRI model. Our data suggests that cardiac cells exhibit similar death kinetics to renal TECs, indicating that ferroptosis may be an important mechanism regulating cardiac allograft survival.

Keywords: ferroptosis, iron, erastin, endothelial cells, regulated necrosis, cardiac allograft model, ischemia reperfusion injury

Poster Abstract #34

Elucidating the Roles of Human Tissue Kallikreins in the Pathogenesis and Behavior of Pleomorphic Adenoma

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Introduction: Pleomorphic adenoma (PA) is the most common salivary gland tumour. This benign tumour is a slowly growing, firm single nodular mass with symptoms of varying duration. PA presents across a wide age range, but is most commonly seen in the 4th to 7th decades with a slight female predilection. Malignant transformation of PA occurs in as many as 5% of cases. Further insight into the pathogenesis of PA may one day be gleaned by analyzing panels of biomarkers such as human tissue kallikreins (KLKs). To better understand the pathologic and physiologic functions of KLKs in PA, we plan to investigate the expression patterns of KLK1–15 at the mRNA level and protein level and compare with normal salivary controls. We hypothesize that various KLKs will be co-expressed and dysregulated in PA.

Methods: To test this hypothesis, we obtained salivary gland PA tissue specimens (N=17) and normal salivary gland controls. The samples were subjected to quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) experiments to detect the mRNA levels of KLK1–15. Statistical analyses were carried out using Wilcoxon signed rank test with the level of significance set at $P<.05$. To corroborate our findings, we plan to assess the protein level expression of KLK1–15 via immunohistochemical staining of all samples.

Results: Preliminary findings revealed expression of mRNA for KLK1–15 in all samples, with a statistically significant decrease in KLK1, 12, and 13 mRNA levels in PA tissues relative to control tissues. We are currently in the process of conducting immunohistochemical analyses of all samples.

Conclusions: We have demonstrated that KLK1, 12, and 13 mRNA levels in PA tissues are statistically significantly decreased relative to those in normal salivary gland control tissues. We have yet to corroborate these findings with immunohistochemical analyses, but experiments are currently underway.

Keywords: Pleomorphic adenoma, salivary gland tumours, human tissue kallikreins, quantitative real-time reverse transcription polymerase chain reaction, immunohistochemical analyses

Poster Abstract #35

Administration of mitochondrial targeted anti-oxidants reduces cardiomyopathy and improves function in diabetic mice

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Introduction: Reactive oxygen species (ROS) production and consequent oxidative stress have been implicated in diabetic cardiomyopathy. Mitochondria are considered as one of main sources of ROS in cardiomyocytes. However, it has never been reported whether selective inhibition of mitochondrial ROS reduces cardiomyopathy in diabetes. This study investigated the therapeutic effects of mitochondria-targeted antioxidants on diabetic cardiomyopathy in both type-1 and type-2 diabetic mice.

Methods: Type-1 diabetes was induced in mice with multiple injections with streptozotocin (STZ, 50 mg/kg/day for 5 days, i.p.). Both type-1 and type-2 (db/db) diabetic mice received mito-TEMPO (0.7 mg/kg/day, i.p.) treatment for 30 days. Mito-TEMPO, a physicochemical compound as one of superoxide dismutase (SOD) mimics, is a mitochondria-targeted antioxidant with superoxide and alkyl radical scavenging properties. One month after treatment with mitochondria-targeted antioxidant, myocardial function was assessed by echocardiography. Cardiomyocytes apoptosis, cardiac hypertrophy, cardiac fibrosis, mitochondrial ROS generation and protein carbonyl contents were determined thereafter.

Results: In both type-1 and type-2 diabetic mice, mitochondrial ROS production, cardiomyocytes apoptosis and protein carbonyl contents were increased. Diabetes also induced myocardial hypertrophy and decreased myocardial function in mouse hearts. Administration mito-TEMPO significantly attenuated myocardial dysfunction , decreased cardiomyocytes apoptosis and protein carbonyl contents, and reduced myocardial hypertrophy as determined by decreased cardiomyocyte size and a reduction in hypertrophic gene expression (ANP and beta-MHC) in both type-1 and type-2 diabetic mice.

Conclusions: Administration of mitochondrial targeted anti-oxidants reduces cardiomyopathy and improves function in both type-1 and type-2 diabetic mice. Thus, selective inhibition of mitochondrial ROS generation may represent an effective therapy for diabetic cardiomyopathy.

Keywords: diabetic cardiomyopathy, mitochondrial ROS, mitochondria-targeted antioxidant, hypertrophy.

Poster Abstract #36

Characterization of cells with high ALDH activity in the human fetal pancreas

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Introduction: ALDH is regarded as a pluripotent marker that regulates differentiation, but determining its contribution to endocrine differentiation is partially undefined. This study aims to characterize transcription factors and stem cell surface markers with ALDH activity in the human fetal pancreas, and examines expansion and differentiation in vitro and in vivo.

Methods: Human fetal pancreata (18 to 22 weeks of fetal age) were obtained based on the approved protocol from the Health Human Sciences Research Ethics Board at Western University and dissociated for fluorescence- activated cell sorting (FACS). Sorted cells with high (ALDHhi) or low (ALDHlo) ALDH activity were either fixed for histological analysis, examined using real-time RT-PCR, or expanded for in vitro culture analysis. Expanded ALDHhi and ALDHlo cells were analyzed for cell lineage markers and transcription factors (TFs) after culture, and after differentiation into clusters, by using histological analysis and real-time RT-PCR. Sorted cells suspended in fibrin gel matrices were also transplanted into nude mice to examine the effects of an in vivo environment on development.

Results: FACS demonstrated high co-localization of the stem cell marker CD133 with ALDHhi cells, while high co-localization of CD34 was observed in the ALDHlo population. Sorted ALDHhi populations also contained higher expression of islet-associated TFs, as well as the islet hormones insulin and somatostatin. Expansion of sorted ALDHhi cells resulted in down-regulation of endocrine TFs genes and ductal CK19, but SOX9 (pancreatic progenitor TF) was still present in ALDHhi cultured groups. Differentiated ALDHhi islet-like clusters restored CK19 and demonstrated increased gene expression of endocrine TFs. Sorted cells transplanted within nude mice demonstrated increased vascularization in ALDHlo populations.

Conclusions: In summary, this study demonstrated that ALDHhi cells contain a CD133- enriched endocrine progenitor cell pool in the developing human pancreas. These results are important for further understanding β-cell differentiation during development.

Keywords: Aldehyde dehydrogenase, CD133, β-cell differentiation, FACS, Human fetal pancreas

Poster Abstract #37

Inducible Beta-Cell Specific β1-Integrin Knockout Affects Islet Architecture, Beta-Cell Survival and Function

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Introduction: It has been shown that β1-integrin is essential for pancreatic beta-cell development and maintenance throughout life in rodents and human fetal islets. However, the effects of a temporarily controlled β1-integrin knockout (β1KO) specific to pancreatic beta-cells of mice in vivo remains to be determined.

Materials and Methods: We have generated C57BL/6 mice with CreER recombinase specific to the mouse insulin promoter (MIP), allowing us to induce a β1KO upon injection of tamoxifen. Male mice at 3-4 weeks of age received 4mg of tamoxifen per 20g bodyweight via intraperitoneal injection for 3 consecutive days. Metabolic studies were conducted by intraperitoneal injection to examine glucose tolerance, insulin tolerance and glucose stimulated insulin secretion. Immunofluorescence staining was used to examine beta-cell mass, islet size and islet density. Pancreatic islets were isolated and protein levels were determined by western blot.

Results: The protein level of β1-integrin in β1KO mouse islets was reduced (~60%) at 8 weeks post induction compared to littermate controls. β1KO mice showed significantly impaired glucose tolerance and glucose stimulated insulin secretion 8 weeks post induction ($p < 0.05$), however insulin sensitivity remained unaltered. Islet morphologic analysis of 8 week post induction β1KO mouse pancreatic sections showed a significant reduction in beta-cell mass, islet density, and number of large islets ($p < 0.05$). We found a significant reduction in Pdx-1, cyclin D1, and c-PARP protein expression, as well as a reduction in p-FAK and p-AKT ($p < 0.05$)

Discussion: Our research shows that β1-integrin is an important regulator of pancreatic beta-cell mass and survival in adult mice. This information could aid in future therapeutic techniques involving islet/beta-cell growth and maintenance in vitro and subsequently in islet or beta-cell transplants in diabetics.

Keywords: diabetes, beta-cells, β1-integrin, mouse insulin promoter, creER recombinase, glucose metabolism

Poster Abstract #38

Protective effect of modified human Fibroblast Growth Factor on Diabetic Nephropathy

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Introduction: Oxidative stress is a key mechanism causing Diabetic Nephropathy (DN). Acidic fibroblast growth factor (aFGF) is known to confer protection from oxidative stress. However, it also has significant angiogenic activity. Hence, we have generated a mutated human acidic FGF (maFGF), with intact antioxidant properties but devoid of angiogenic activities. We hypothesized that maFGF treatment has a protective effect in DN.

Methods: We aimed to investigate the effects of maFGF treatment on the biochemical, structural and functional changes in a mouse model of type I diabetes. Streptozotocin induced diabetic mice were treated with maFGF (IP injection) daily for 1 month or 6 months. They were compared with untreated diabetic animals and non-diabetic controls. Functional changes in the kidneys were determined using urine albumin to creatinine ratio. For mechanistic studies, conditionally immortalized mouse podocytes and human microvascular endothelial cells were exposed to high glucose (25mM) or low glucose (5mM). Oxidative stress, DNA damage and extracellular matrix proteins were assessed through real-time qPCR and ELISA or Western blot. Glutathione peroxidase and catalase activity assays, immunohistochemical staining and histologic analyses were performed. Reactive oxygen species (ROS) levels and nitric oxide (NO) production were assessed to examine possible mechanisms.

Results: MaFGF treatment did not affect body weight and blood sugar levels. maFGF, however prevented renal functional alterations in diabetes at both time points. It also prevented diabetes –induced DNA damage, upregulation of angiotensinogen and oxidative stress marker heme oxygenase 1. Although it failed to prevent alterations of the fibrogenic factor TGF β1 mRNA expression, it showed some preventive effects on diabetes induced extracellular matrix protein upregulation. Further analyses show that such prevention is mediated through maFGF induced prevention of DNA damage via alteration of nitric oxide production.

Conclusion: Data from these experiments indicate a potential therapeutic role of maFGF in diabetic nephropathy.

Keywords: acidic FGF, modified acidic FGF, diabetic nephropathy, oxidative stress, nitric oxide.

Poster Abstract #39

Diabetic Marrow Adipogenesis Alters Composition of Stem Cell Niche and Impairs CD133-positive Stem Cell Survival

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Introduction: Diabetic hyperglycemia enhances the adipogenic differentiation of bone marrow (BM) mesenchymal progenitor cells (MPCs). Within the BM also reside vascular stem cells (VSCs) that contribute to endothelial repair. Defects in the proliferation, differentiation, and function of VSCs have been noted in diabetes and contribute to the development of vascular complications. We believe the skewed developmental potential of MPCs in diabetes alters the composition of the BM stem cell (SC) niche in a way which impairs the survival of CD133-positive stem cells, with CD133 serving as the best marker at present of VSCs.

Methods: BM-derived MPCs, and adipocytes and osteoblasts differentiated from MPCs were cultured with a population of CD133-positive SCs. We then assayed for SC-specific markers to examine phenotypic changes. qPCR was them employed to profile the differences in the expression of extracellular, secreted, and cell surface niche proteins by adipocytes, osteoblasts, and MPCs that may contribute to the altered BM SC niche seen in diabetes.

Results: Following co-culture with adipocytes, a significant reduction in the survival of CD133 SCs was observed relative to culture with MPCs or osteoblasts. We observed selective modulation of ECM remodelling genes, as well as secreted and surface niche factors. We next tested the effects of two ECM component proteins on cell differentiation. While collagen I appeared to have no significant effect on adipogenesis, culture of MPCs on fibronectin-coated plates delayed differentiation.

Discussion: Our findings suggest that adipocytes are responsible for the creation of a distinct microenvironment that may influence SC survival and development. Altered BM-SC function and differentiation may mediate the connection between enhanced BM adipogenesis, SC depletion, and impaired vascular repair in diabetes. Preventing the diabetes-induced changes to the composition of the BM may present a novel strategy to employ in preventing or managing the vascular complications of this disease.

Keywords: diabetes, bone marrow, adipogenesis, mesenchymal progenitor cells, vascular stem cells

Poster Abstract #40

Hematopoietic progenitor phenotype induced by T-box 2 knockdown in infantile hemangioma cells

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Introduction: Infantile hemangiomas (IH) are benign vascular neoplasms characterized by the differentiation of multipotential stem cells (hemSCs) into endothelial cells during the early proliferation phase, and later into adipocytes during spontaneous involution. Our laboratory has identified elevated T-box 2 (TBX2) activity in hemSCs and shown an important role in adipogenic differentiation.

Hypothesis: I hypothesize that TBX2 is a regulator of infantile hemangioma development, and maintains a differentiation-competent state in hemSCs. Based on the role of TBX2 in adipogenesis, I expect TBX2 represses other lineages and orchestrates a mesodermal fate.

Methods: HemSCs isolated from human IH lesions were characterized for TBX2 expression through mRNA analysis and immunofluorescence staining. The ability of hemSCs to differentiate into mesenchymal, endothelial, neurogenic, and hematopoietic cells was evaluated to determine whether levels of TBX2 correlate with differentiation competence. TBX2 knockdown through RNAi was performed in hemSCs followed by culture in lineage specific induction media. Changes in differentiation of hemSCs was evaluated by assessing cell markers specific for each lineage.

Results: My results show that TBX2 is localized primarily in the nuclei of hemSCs; the level of expression varied between hemSC cultures of different patient-derived hemangiomas. HemSC cultures were capable of differentiating towards mesenchymal, endothelial, and neurogenic lineages. Upon TBX2 knockdown, hemSCs exhibited upregulated expression of CD34 (endothelial and hematopoietic marker), CD45 (hematopoietic marker), and ckit (stem cell marker), as well as produced burst-forming unit colonies, indicating erythropoiesis. Interestingly, hemSCs without TBX2 knockdown did not yield hematopoietic colonies.

Conclusion: My studies show that TBX2 knockdown induces a hematopoietic progenitor phenotype suggesting it potentially represses hematopoietic differentiation in hemSCs. Understanding these mechanisms may provide new treatment targets for infantile hemangioma and may also provide insight into hematopoietic progenitor cell differentiation.

Keywords: Infantile hemangioma, T-box 2, hematopoiesis, stem cell, differentiation.

Poster Abstract #41

Etiology of Motor Vehicle Collision Fatalities in Southern Ontario

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Factors such as alcohol use, speed, restraint use, and driving experience have been identified as potential risks for fatalities in motor vehicle collisions. Environmental factors including weather conditions, road type, and temperature have also been associated with motor vehicle collision fatalities. The current study evaluates whether human, environmental, and vehicular factors act individually to cause motor vehicle collision mechanisms that increase the probability of an occupant fatality in a motor vehicle collision in southern Ontario. Data will be collected and analyzed from a database of motor vehicle collisions compiled by the Motor Vehicle Safety (MOVES) Research Team based at Western University. The database includes observations from the post-collision scene, vehicular damage, and Event Data Recorder records from the case vehicle. Medical data that documents fatal and nonfatal injuries has also been collected and included in the database. Statistical analyses will be used to simultaneously analyze multiple potential risk factors involved in motor vehicle collisions in order to identify factors that significantly contribute to the probability of a fatality of an occupant involved in a motor vehicle collision. These analyses will allow for an interpretation of the results that allows individual factors to be correlated to different potentially fatal collision mechanisms. Subsequent analyses will allow for the “fatality potential” of individual factors to be compared and ranked. Investigation of human, environmental, and vehicular factors involved in the motor vehicle collisions will allow for individual collision mechanisms and injury mechanisms to be correlated with the particular factors. This allows for the elucidation of a comprehensive narrative that details motor vehicle fatalities in relationship to the factors that promote and precede the collisions.

Keywords: Forensic Pathology, Motor Vehicle Collision, Motor Vehicle Occupant Trauma, Collision Analysis, Postmortem examination, Accident Prevention

Poster Abstract #42

Implementation of the serum/ plasma methylmalonic acid test at the London Health Sciences Centre

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Introduction: Methylmalonic acid (MMA) is the free acid form of methylmalonyl-CoA, an intermediate in the conversion of the breakdown products of various macromolecules to succinyl-CoA, which enters the Krebs cycle. Levels of methylmalonic acid in serum or plasma can be measured, and are used in the diagnosis of diseases such as vitamin B12 deficiency and congenital methylmalonic acidemia. In this study, we attempted to implement the serum/ plasma MMA test by gas-chromatography/mass spectrometry (GC/MS) in the Toxicology/TDM Lab at LHSC.

Methods: The mass spectra of MMA and its trideuterated analogue were obtained using a standard scan program in the GC/MS instrument. A literature-based method was then programmed into the instrument, and the initial sample preparation steps were modified to optimize the sensitivity and peak shapes of the analytes. Horse serum-based standards were used to establish a calibration curve that was used in the calculation of MMA levels in patient samples.

Results: The calibration curve was linear, and the endogenous level of MMA in the horse serum was calculated to be approximately 400nmol/L. The upper quality control samples (550nmol/L) exhibited acceptable values of within-day and total coefficient of variation that were under 15%, while the lower quality control samples (320nmol/L) showed more variability. The MMA levels in 10 of 12 randomly selected patient samples tested were in the reference range (100-400nmol/L), with two samples somewhat higher.

Conclusions: The complexity and difficulty associated with the setup of the test were demonstrated by various problems with both the instrument and the sample preparation methods – both the GC/MS settings and the initial preparation procedure were altered during the duration of the project. We did not reach the point where we could perform correlation experiments with patient samples that hand been previously sent to the reference lab for analysis, and the test will have to be further developed.

Keywords: Methylmalonic acid, vitamin B12 deficiency, congenital methylmalonic acidemia, gas chromatography, mass spectrometry, isotope dilution

Poster Abstract #43

Targeting T memory cells with Receptor Interacting Protein Kinase 3 (RIPK3) mediated Necroptosis

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Introduction: Post-operative graft rejection is a risk that cardiac transplant patients live with for the rest of their lives. Hence, post-operative drug regimens include immunosuppressive drugs to prevent allograph rejections. The use of immunosuppressive drugs also poses a risk for the patient in terms of immunity. The current goal is to find a novel immunomodulatory strategy, specifically by inducing cell death of T memory cells involved in graft rejection to prevent rejections. The key player in graft rejections are T memory cells involved in the immune response against the foreign organ. Inducing T memory cell death remains elusive, due to the anti-apoptotic quality of T memory cells. We hypothesize that the RIPK3-mediated necroptotic pathway can be used to induce T cell death.

Methods: CD4+ T cells and CD8+ T cells of the Balb/C mouse background will be cultured from the spleen and lymph node and activated by mixed lymphocyte reaction (MLR) using B6 splenocytes. After 5 days, we will induce TNF-mediated necroptosis. The level of death and type of the death will be measured using flow cytometry, immunohistochemical staining, and immunoblot for levels of high mobility group box 1 protein (HMGB1). We will repeat this using T memory cells.

Results: Our results show that TNF-mediated necroptosis can be induced in CD4+ and CD8+ T cells by using TNFα, SMAC mimetics and Z-VAD-FMK and reduced by using Necrostatin-1 to inhibit RIPK3.

Conclusion: The findings show that the death of CD4+ and CD8+ T cells can be manipulated. The results provides us with the conditions required to induce necroptosis in T cells.

Keywords: Graft rejections, T memory cells, apoptosis, necroptosis, necrosis, RIPK3, TNFα-mediated cell death, cardiac transplants

Poster Abstract #44

Effect of Mechanical Stretch on Extracellular Matrix of Human Trabecular Meshwork Cells

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Introduction: Open-angle glaucoma is a result of excess accumulation of extracellular matrix (ECM) within the trabecular meshwork (TM). This prevents proper outflow of the aqueous humor, resulting in elevation of intraocular pressure (IOP) and optic nerve damage. It is known that phosphatase and tensin homolog (PTEN) is a major regulator of ECM remodelling, and increased mechanical strain on the TM induces deposition of excess ECM. Since IOP elevation resulting from glaucoma increases mechanical stretch on the TM, we have investigated the changes in expression of PTEN as well as changes in ECM with application of physiological and pathological mechanical stretch on human TM cells. We hypothesize that mechanical stretch on human TM cells modulates expression of PTEN. This regulation of PTEN in turn modulates the deposition or degradation of ECM.

Methods: Human TM cells from embryonic donors were subjected to physiological (5%) and pathological (15%) mechanical stretch using the FX5000 Tension System at 1Hz for 24 and 48 hours. Proteins were then extracted and analyzed for the expression of PTEN and collagen using immunoblot.

Results: Our results show that there is a significant elevation in the expression of type I collagen by TM cells as mechanical stretch is increased in duration (24 to 48 hours) and magnitude (5% to 15%). At 15% stretch for 48 hours, expression of PTEN decreased, and this was associated with higher levels of collagen in ECM.

Discussion: Our results indicate that pathologic stretch of TM, as in patients with glaucoma, decreased the expression of PTEN and thus could be a factor in the accumulation of excess ECM in TM. This finding signifies that increasing the activity of PTEN could be a valuable therapeutic target to treat glaucoma by decreasing ECM deposition in TM and thus reducing IOP.

Keywords: glaucoma, mechanical stretch, trabecular meshwork, intraocular pressure, PTEN

Poster Abstract #45

Feasibility of targeting PIK3CA mutations in head and neck squamous cell carcinoma

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Introduction: PIK3CA is a frequently mutated, directly druggable oncogene in head and squamous cell carcinoma (HNSCC) which holds promise as a therapeutic target. However, clinical trials of therapeutics targeted against PIK3CA activating mutations require prompt molecular testing to identify treatment candidates. This study aims to evaluate 1) local prevalence of PIK3CA activating mutations in HNSCC, 2) timeliness of our mutation-profiling pathway, and 3) patients' willingness to enroll in a neoadjuvant drug trial.

Methods: Twenty-five consecutive cases of newly diagnosed HNSCC from the London Regional Cancer Centre catchment area were identified. DNA was extracted from the pretreatment biopsies and tested for PIK3CA activating mutations at three mutational hotspots by real-time PCR. The prevalence of PIK3CA activating mutations and the turnaround time between case identification and PIK3CA mutational status determination were calculated. 30 HNSCC patients were surveyed prospectively regarding willingness to participate in a hypothetical drug trial. Survey data was summarized descriptively.

Results: 4 of 25 (16%) tumors harbored a PIK3CA activating mutation, including one at codon E542K, two at codon E545K/D, and one at codon H1047R. On average, this result was obtained within approximately 15 working days. Important factors that increased turn-around time included shipment delays in receiving specimens from outside centres and delays associated with implementation and optimization of the PIK3CA PCR assay within our centre. 70% of patients surveyed indicated their willingness to participate in a targeted PIK3CA trial.

Discussion: This study demonstrates that PIK3CA activating mutations can be detected with expected prevalence and with sufficient timeliness to mount a targeted intervention trial, and there is a patient willingness to participate. Additionally, key sources of delay were identified enabling further improvements in turnaround time. This study paves the way for molecularly-driven therapeutic studies that may lead to improved tumor response and decreased treatment-related toxicities in selected HNSCC patients.

Poster Abstract #46

The protective role of Junctophilin-2 in Ischemia-reperfusion injury

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Introduction: Reperfusion therapy which can restore blood flow into ischemia myocardium is a main treatment against acute myocardial infarction. However, from the onset of reperfusion it initiates further disruption of cardiomyocytes and lethal cell injury. Aggravated calcium overload and mishandling after reperfusion had been proved as a key role lead to cardiac arrhythmias and cell death. Junctophilin-2(JPH2) as a junctional membrane-binding structural protein mechanically maintain the fixed distance between T-tubule and sarcoplasmic reticulum(SR), allowing the proper Ca2+ induced Ca2+ release for stable excitation-contraction coupling. Recent studies showed that JPH2 also regulate the gating of ryanodine receptor 2(RyR2) and the activation of Na+/Ca2+ exchanger (NCX).Since the crucial connection between cellular calcium handling and JPH2 and its reported down-regulated in hypotrophy and heart failure model, we hypothesize that over-expression of Junctophilin-2 can protect the cardiomyocytes from ischemia-reperfusion induced cell death via altering Ca2+ overload.

Method: To test this hypothesis, H9C2 cultured cells were subjected to hypoxia and re-oxygenation(H/R) by using the GENbag anaer. Adenovirus infection was utilized to over express JPH2. We then tested the Apoptosis by Caspase-3 assay and Cellular DNA Fragmentation ELISA. We also determined the JPH2 expression and ER stress by Western blot. Presently we are repeating the whole procedure on isolated neonatal cardiomyocytes.

Results: In H9C2 cells, apoptosis is highly induced over 24h/24h hypoxia/re-oxygenation and in the meantime JPH2 is down regulated. Time-course show that these changes are all triggered after the onset of reperfusion. We also successfully over expresses JPH2 by using adenovirus infection and find out under the same H/R condition its apoptosis value significantly drops down. One of the ER stress connected protein Phosphate JNK also decrees.

Conclusions and future directions: These previous findings demonstrate that over-expression of JPH2 can protect the H9C2 cells from hypoxia/re-oxygenation induced apoptosis. We are working on the isolated neonatal cardiomyocytes and this model shows great potential of similar results. We will soon begin to examine the Ca2+ changes under H/R and whether the over-expressed JPH2 will improve the Ca2+ handling and alter calcium overload. Next we will try to decipher the mechanisms especially the interesting connection in JPH2, ryanodine receptor and NCX. In the future we will move our study to in vivo by adopting transgene mice or rats model and surgical ischemia-reperfusion procedure. Other kinds of cell death will be also determined

Key words: Junctophilin-2, Ischemia-reperfusion injury, Ca2+ overload, apoptosis,

Poster Abstract #47

Elucidating the Role of RGNEF and Binding Partners in Neuronal Development

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Introduction: Our lab recently discovered RGNEF: the human homologue to the low weight neurofilament (NFL) mRNA stabilizing factor p190RhoGEF. In addition to interacting with NFL mRNA, p190RhoGef has been shown to interact with δ-Catenin to influence dendrogenesis; a site necessary for δ-Catenin-p190RhoGef has been found on δ-Catenin but not p190RhoGEF. We hypothesize that the δ-Catenin - p190RhoGef interaction is mediated by one or more mutual binding partner. Additionally, given p190RhoGef's activity as a GEF, we intend to explore the binding partners necessary for p190RhoGef's localization to the plasma membrane; two amino acids in the PH Domain have already been implicated

Methods: RGNEF, the human homologue to p190RhoGef, binding partners specific for localization will be identified in HEK293T cell culture through the use of cell fractionation and immuneprecipitation (IP) followed by both 2D IEF and mass spectrometry. Results will be confirmed using IHC staining and confocal microscopy. Additionally, mutant RGNEF lacking PH Domain (ΔPH) will be examined to elucidate the specific role the PH domain plays both in localization and binding partners.
We will also analyze the p190RhoGef - δ-Catenin interaction in mouse primary neuronal culture to determine the roles p190RhoGEF binding partners, localization, and interaction plays in the neuron. Having identified p190RhoGef specific binding partners, we will again use immuneprecipitation to identify both δ-Catenin specific and δ-Catenin - p190RhoGef mutual binding partners via 2D IEF. These results will be confirmed by mass spectrometry and interactions confirmed by co-IP.

Results: We predict there will be multiple PH domain specific binding partners for RGNEF and that at least one of these proteins will play a necessary and sufficient role in the localization of RGNEF to the plasma membrane. The identification of these bidning partners will elucidate the role that RGNEF plays in cellular function
In neurons, we expect to find one or more δ-Catenin - p190RhoGef mutual binding partner and that the previously described p190RhoGef binding domain within δ-Catenin is in fact specific for these binding partners.

Conclusions: Given its function as both a GEF and RNA binding protein, these data will identify specific pathways to pursue to clarify RGNEF's function. We will have also examined the δ-Catenin-p190RhoGef complex, including associated proteins, and thus identified possible activities involved in dendrogenesis and neurological diseases.

Poster Abstract #48

Variable Region Sequencing of a Monoclonal Antibody Against Escherichia coli O157.

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Introduction: Monoclonal antibodies (mAbs) have become a valuable material not only for therapeutics, but also as diagnostics tools, due to their high specificity and unlimited production capacity through hybridoma technology. Their vast repertoire relies on their variable regions of the heavy- and light-chains, specifically the antigen-binding sites. Thus, obtaining the variable domain nucleotide sequence has become an adequate approach to understand the uniqueness and complexity of mAbs. In this study, we attempted to sequence the variable regions of a mAb developed for targeting the O157 antigen found in pathogenic E. coli cells. We hypothesized that by using particular degenerate primers we would be able to amplify and direct sequence the heavy- and light-chains variable regions.

Methods: Hybridoma cells producing the anti- E. coli O157 mAb were cultured, sub-cloned and expanded. The supernatant was collected for antibody isotyping, while the isolated hybridoma cells were used for RNA extraction. Furthermore, RNA was reverse transcribed and the cDNA was amplified using highly degenerate primers for both, heavy- and light-chain variable regions. The amplification products were resolved by gel electrophoresis and stained with ethidium bromide. The bands of the appropriate sizes were excised, purified and sequenced directly. Finally, the variable region sequences were aligned and compared with previously published mouse immunoglobulin nucleotide sequences using the BLAST algorithm.

Results: So far, we have obtained the nucleotide sequence of the heavy-chain variable region, compared it using the BLAST algorithm and identified some of the genes that encode this domain.

Conclusions: The degenerate primers selected for this study have successfully amplified the monoclonal anti- E. coli O157 antibody variable domains. In addition, we were able to obtain a specific sequence, which was confirmed to belong to the heavy-chain variable domain of a mouse mAb by using the BLAST algorithm.

Keywords: E. coli O157, sequencing, mouse monoclonal antibody, degenerate primer, variable region, BLAST.

Poster Abstract #49

Mechanisms Underlying Excess Matrix Deposition in the Trabecular Meshwork in Glaucoma

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Introduction: Glaucoma is the second leading cause of irreversible blindness world-wide. It is postulated that increased extracellular matrix (ECM) deposition in the trabecular meshwork (TM) tissue decreases aqueous humor (AH) outflow, leading to increased intraocular pressure (IOP) and subsequent optic nerve damage that is characteristic of glaucoma. Transforming growth factor-beta (TGFβ2) is a well-known inducer of ECM deposition and levels of active TGFβ2 have been shown to be increased within the AH of glaucoma patients. Recently it has been established that protein phosphatase and tensin homolog (PTEN) has a major role in regulating ECM deposition. In dermal fibroblasts, overexpression of PTEN decreases collagen deposition, whereas decreased PTEN expression leads to increases in collagen deposition. The role of PTEN in the TM of normal and glaucomatous eyes is not yet known. We hypothesize that TGFβ2 modulates the deposition of ECM in the TM by regulating the expression of PTEN, ultimately affecting aqueous outflow.

Methods: Human TM cells were cultured and treated with TGFβ2 and small molecule inhibitors to downstream TGFβ signaling were used to detect pathways that control PTEN expression. Protein was extracted and expression levels of PTEN and other signaling molecules were analyzed using immunoblots.

Results: Addition of TGFβ2 increased expression levels of PTEN and TGFβ related signaling pathways. Further experiments are planned to use inhibitors of TGFβ signaling to delineate pathways that control the expression of PTEN.

Conclusions: These results show some of the effects of TGFβ2 on PTEN expression. Mechanisms by which TGFβ signaling modulates PTEN expression in TM cells need to be studied in greater detail. This study could establish the maintenance of normal PTEN signaling as an effective therapeutic strategy to inhibit excess deposition of ECM in the TM, preventing increase in IOP and vision loss in glaucoma.

Keywords: Glaucoma, trabecular meshwork, PTEN, excess ECM deposition, fibrosis, aqueous outflow resistance, TGFβ2

Poster Abstract #50

Role of Long Non-Coding RNAs in Diabetic Complications

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Introduction: In this project we will investigate lncRNAs— H19 and ANRIL to elucidate their specific roles in cellular and extracellular changes during diabetic complications. We have previously shown that histone acetylator,p300 regulated microRNA-200b regulates glucose-induced VEGF production in diabetes. Here we examined if H19 and ANRIL are altered in diabetic complications.

Hypothesis: LncRNAs— H19 and ANRIL regulates extracellular and vasoactive factors in diabetic complications through miR-200b and/or p300.

Materials and Methods: In this study, we plan to elucidate interactions of lncRNAs— H19 and ANRIL with miR-200b and histone acetylator, p300. These alterations will be studied using multiple models. In vitro experiments will utilize endothelial cells (ECs), retinal cells, podocytes and cardiomyocytes exposed to hyperglycemia. In vivo models will include STZ mice models for chronic type1diabetes and db/db for type 2 diabetes. We will perform siRNA experiments to understand the mechanistic roles of these lncRNAs and decipher their interaction with miR-200b and p300 in diabetic conditions.

Results: ECs exposed to high glucose decreased the expression of H19 and enhanced ANRIL expression. This was accompanied by elevation of vasoactive factors like VEGF and ECM proteins such as Fibronectin. These factors were however reduced in expression on downregulation of ANRIL and H19 lncRNAs. The expression levels of H19 and ANRIL were similarly altered in retina, heart and kidneys of diabetic mice compared to controls. We hope to further elucidate their roles in diabetic complications, through overexpression of these lncRNAs and STZ-induction in H19 and ANRIL-specific mouse models.

Conclusions: The altered levels of H19 and ANRIL in ECs and tissue samples of diabetic mice indicate their potential downregulation and elevation, respectively during diabetic complications. We hope to explicate their regulatory functions through potential interactions with miR-200b and p300.

Keywords: H19, mir-200, p300, acetylation, diabetic complications

Poster Abstract #51

Upregulation of ATP Synthase 5A1 ameliorates calpain-mediated mitochondrial ROS generation and diabetic cardiomyopathy

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Introduction: Cardiomyopathy is arguably the most concerning complication of diabetes. It is characterized by the apoptosis, hypertrophy, and fibrosis of cardiac muscle. The protease calpain is an important mediator of diabetic cardiomyopathy. Our lab has previously shown that cardiomyocytes isolated from diabetic mice display increased calpain activity. The current study aims to investigate a specific pathway by which calpain inhibits the ATP synthase 5A1 subunit in the mitochondria to generate reactive oxygen species (ROS) and induce cardiomyopathy. As well, the potential for ATP synthase 5A1 upregulation to serve as a therapy will be investigated.

Methods: A diabetic mouse model was created using streptozotocin (STZ), and the heart was surgically isolated once diabetes was successfully induced. Calpain activity in the mitochondria was measured using the fluorescent substrate N-succinyl-LLVY-AMC, and ROS level in the mitochondria was measured using Amplex® Ultrared. Interaction between calpain and ATP synthase 5A1 was shown using immunoprecipitation. Upregulation of ATP synthase 5A1 was achieved using adenovirus transfection. Finally, fluorescence microscopy and the ImageJ software was used to measure the degree of cardiomyopathy, based on the extent of hypertrophy and fibrosis.

Results: Our results show that calpain activity and ROS level are both elevated in the mitochondria of diabetic cardiomyoctes. This is mediated through ATP synthase 5A1 inhibition, since overexpression of the ATP synthase 5A1 gene resulted in decreased ROS generation, hypertrophy, and fibrosis. This is further confirmed by immunoprecipitation results showing interaction between calpain and ATP synthase 5A1.

Conclusions: Our findings suggest that diabetes results in increased mitochondrial calpain activity and inhibition of ATP synthase 5A1 subunit, ultimately leading to diabetic cardiomyopathy. These findings reveal a specific pathway of the disease and propose a potential therapy.

Keywords: diabetes, cardiomyopathy, calpain, ATP synthase 5A1, cardiomyocytes, ROS

Poster Abstract #52

Effects of the Western diet and IUGR on Glucose Homeostasis

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Introduction: Intrauterine growth restriction (IUGR) induced by uteroplacental insufficiency has been shown to predispose individuals to visceral obesity and type 2 diabetes. Similarly, a diet high in saturated fat and fructose, characteristic of the Western diet, has been linked to the development of obesity and type 2 diabetes. Previous studies done in humans and rodents, indicate that both can increase the risk of altered lipid homeostasis, insulin resistance, and β-cell dysfunction in late adulthood. The current study will examine the changes in the morphology of the endocrine pancreas by the single or combined treatment. We hypothesize that a Western diet in combination to placental insufficiency will further alter pancreatic development and predispose individuals to glucose intolerance earlier in life.

Methods: Guinea pigs were used due to their development of a more mature pancreas at birth. Normal birth weight and low birth weight (induced by uterine artery ablation) pups were either fed a control diet or a Western diet until sacrifice. Pancreata were collected at postnatal day 145 and tissues were examined by single or dual immunohistochemistry to detect glucagon and insulin. Microphotographs were taken to determine the number of islets, size of islets, α-cell area, β-cell area, and total islet area.

Results: The results will show that the presence of IUGR and a Western diet, individually and in combination, will cause a decrease in the number of islets, α-cell area, β-cell area, and total islet area as well as a differing distribution of islet size in comparison to their respective controls.

Conclusions: These findings show that dietary exposure affects the morphology of the endocrine pancreas, that IUGR may affect the pancreas development in utero and this may affect islet morphometry later in life and that the combined effect of a Western diet and IUGR will increase the risk of β-cell failure earlier in life.

Keywords: Uteroplacental insufficiency, intrauterine growth restriction, Western diet, β-cell, type 2 diabetes

Poster Abstract #53

Mechanisms by which the Pancreatic β-cell Insulin Receptor Regulates βcell Growth, Function and Survival

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Introduction: Insulin receptor expression in β-cells suggests that insulin has an autocrine/paracrine role in the regulation of β-cell function. Previous studies have demonstrated that β-cell Ir knockout mice develop age-dependent glucose intolerance, impaired insulin secretion, and eventual β-cell atrophy. However, temporally controlled βIrKO experiments have yet to be investigated in vivo.

Methods: We utilized mouse insulin 1 promoter driven tamoxifen-inducible Cre-recombinase Ir knockout mouse model to perform temporal β-cell specific Ir knockout. For prenatal and neonatal studies, pregnant mice received an intraperitoneal (IP) injection of 6mg/40g of BW tamoxifen at E13. Mice were sacrificed and pancreata were obtained either at fetal E19 or neonatal P21 for morphology analyses. For postnatal and HFD studies, adult mice received 3 IP injections of 4mg/20g of BW tamoxifen at 3~4weeks of age. Pre-diabetic experimental groups were fed HFD for 6 to 18 weeks. We conducted in vivo IP glucose tolerance tests, IP insulin tolerance tests, and glucose stimulated insulin secretion at 12 and 24 weeks of age. Pancreata will then be dissected for morphological analysis and activity of downstream Ir signaling pathways.

Results: Compared to controls, fetal βIrKO mice displayed a significant decrease in the percentage of Ir+ β-cells ($p<0.001$). Morphologically, βIrKO mice exhibited increased: islet density, mean islet area, total islet area and β-cell area ($p<0.01$). The percentage of large islets (>10 000 um2) was higher in βIrKO pancreata than that of controls ($p<0.05$). βIrKO mice also had increased β-cell proliferation ($p<0.01$) with no change in apoptosis. We demonstrated a compensatory upregulation of Igf-2 protein expression ($p<0.01$), along with increased Akt activity($p<0.05$).

Discussion: Our results demonstrate a developmental role for the β-cell Ir, whereby its loss induces an islet compensatory response that is similar to early type 2 diabetes pathogenesis. Postnatal evaluation of adult βIRKO metabolic and histological phenotypes is under investigation.

Keywords: β-cells, insulin receptor, MIP-CreER, diabetes, tamoxifen, Akt

Poster Abstract #54

Role of plasma osteopontin as a biomarker in locally advanced breast cancer

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Introduction: Osteopontin (OPN), a malignancy-associated secreted phosphoprotein, is a prognostic plasma biomarker for survival in metastatic breast cancer patients. We evaluated the role of OPN in Locally Advanced Breast Cancer (LABC) patients in predicting response to neoadjuvant chemotherapy and association with survival.

Methods: Fifty-three patients with non-metastatic LABC were enrolled in this study and monitored serially for plasma OPN levels by ELISA during neoadjuvant chemotherapy prior to surgery. For fifty patients who had baseline OPN levels available for analysis, the median baseline OPN level was 63.6 ng/ml.

Results: Median patient follow up was 45 months and thirteen patients died from metastatic disease. Patients with baseline OPN levels ≥ 63.6 ng/ml were significantly more likely to die of their disease than those with baseline OPN < 63.6 ng/mL (Hazard Ratio = 3.4; 95% confidence interval 1.4-11.3; P = 0.011), and overall, baseline OPN level was significantly associated with survival (P = 0.002).

Discussion: There was little support for value of serial OPN determination in monitoring response to therapy in this patient population. Although the percentage of patients with baseline OPN levels < 63.6 ng/ml was higher in patients with complete response than in those with no response, the difference was not statistically significant (64% and 14%, respectively (P = 0.066). Thus, baseline plasma OPN level is a prognostic biomarker in this group of LABC patients, and could also be helpful in identifying LABC patients who will respond to neoadjuvant chemotherapy. Our results call for validation of our findings in large prospective trial data sets.

Keywords: locally advanced breast cancer, neoadjuvant therapy, osteopontin, prognostic biomarker, predictive biomarker

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